Box-Behnken Experimental Design in the Development of a Nasal Drug Delivery System of Model Drug Hydroxyurea: Characterization of Viscosity, In Vitro Drug Release, Droplet Size, and Dynamic Surface Tension

Submitted: August 11, 2005; Accepted: October 31, 2005; Published: November 17, 2005

Pankaj Dayal,[1] Viness Pillay,[2] R. Jayachandra Babu,[1] and Mandip Singh[1]

[1]Division of Pharmaceutics, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307

[2]Division of Pharmaceutics, Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa

ABSTRACT

The purpose of the research was to investigate the changes in physicochemical properties and their influence on nasal formulation performance using 5-factor, 3-level Box-Behnken experimental design on the combined responses of viscosity, droplet size distribution (DSD), and drug release. Gel formulations of hydroxyurea (HU) with surface-active polymers (hydroxyethylcellulose [HEC] and polyethylene-oxide [PEO]) and ionic excipients (sodium chloride and calcium chloride) were prepared using Box-Behnken experimental design. The rheology and dynamic surface tension (DST) of the test formulations was investigated using LV-DV-III Brookfield rheometer and T60 SITA tensiometer, respectively. Droplet size analysis of nasal aerosols was determined by laser diffraction using the Malvern Spraytec with the InnovaSystems actuator. In vitro drug release studies were conducted on Franz diffusion cells. With PEO gel, calcium chloride increased the viscosity and DSD and retarded drug release, while sodium chloride decreased the viscosity, DST, and DSD and accelerated the release of HU. With HEC gel, the addition of the above salts resulted in less significant changes in viscosity, DSD, and DST, but both salts significantly increased the release of HU. Droplet size data obtained from a high viscosity nasal pump was dependent on type of polymer, polymer-excipient interactions, and solvent properties. The applications of Box-Behnken experimental design facilitated the prediction and identified major excipient influences on viscosity, DSD, and in vitro drug release.

KEYWORDS: hydroxyurea, viscosity, dynamic surface tension, Box-Behnken experimental design, nasal delivery.

Corresponding Author: Mandip Singh, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307. Tel: (850) 561-2790; Fax: (850) 599-3347; E-mail: mandip.sachdeva@famu.edu

INTRODUCTION

In the development of a nasal drug delivery system (NDDS), formulation characteristics and device capabilities must be harmonized in order for consistent delivery into the nasal cavity. The approach to improve nasal bioavailability is the use of polymeric gel vehicles to increase nasal residence times and to control the rate of drug absorption. The aerosol droplet size distribution (DSD) is an important variable in defining the efficiency of aerosolized drugs. Low viscosity or shear-thinning vehicle systems were effectively atomized into small droplets using different nasal pump sprays, as previously reported.[1] There have been many reports that solutions of mixtures of certain polymers, surfactants, and excipients can exhibit molecular interactions that affect the rheological and physicochemical properties of the solutions.[2] The nature of these interactions can affect the ability of the solution to be aerosolized into small droplets and may alter the stability and liberation of the active components.

In the pharmaceutical industry, polymers are routinely used in the formulation of gels and in the stabilization of emulsions. The stabilization results from the properties of the polymer that demonstrate interfacial properties similar to the actions of surfactants. Polymers are usually large molecules and are used extensively as vehicles to control the release of active components. However, reports on dynamic surface tension (DST) and rheological behavior of nasal aerosols with polymeric vehicles have been limited. A multiple component polymeric nasal formulation represents a complex system in which DST, interfacial, and rheological properties will influence the droplet size generated from nasal devices. The polymer and excipient concentration of the formulation, ionic nature, molecular weight, characteristic diffusion time, interfacial deformation and mobility, and excipient interactions (polarity, complexation, ionic character among others) will influence both the release of the active components from polymeric vehicles and their ability to form small droplets. Owing to the complexity of these interactions, the conventional approach of changing one formulation variable at a time and studying the effect of each variable on the droplet size and/or drug release

behavior is a complex process, particularly in a multivariate system or if more than one response is of importance.

Design of Experiments (DOE) is a statistical technique that can be used for optimizing such multivariable systems. In recent years, the pharmaceutical industry has used experimental designs more for the optimization of pharmaceutical agents; however, only a few are reported in the literature for the development of dosage forms.[3,4] In this investigation, we applied Box-Behnken design to study the effects of formulation components on (1) in vitro drug release of a model drug hydroxyurea (HU), (2) changes in rheology, and (3) DSD generated from a high viscosity nasal pump. We employed 2 nonionic hydrophilic polymers, hydroxyethylcellulose (HEC) and polyethylene oxide (PEO) as gelling agents. Electrolytes can affect the polarity of a solution as well as alter the release of the drug via complexation, which may involve the redistribution of electrostatic bonding between components of a formulation. In addition, electrolytes have been reported as rheological modifiers in polymeric solutions.[5,6] Therefore, calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were employed as model ionic excipients in the polymer gel vehicles. DST studies were also undertaken to elucidate formulation interactions and address different issues related to polymer interfacial properties.

Materials and Methods

Materials

Polyethylene oxide (PEO-1Z-approximate molecular weight of 150 000-400 000) and hydroxethylcellulose (HEC-Natrosol, 250L, *National Formulary* [*NF*]) were donated by Sumitomo Seika (Tokyo, Japan) and Aqualon (Wilmington, DE), respectively. Hydroxyurea (HU) and dialysis membranes were purchased from Sigma-Aldrich (St Louis, MO) and Fisher Scientific (Suwanee, GA), respectively. A high viscosity nasal pump for droplet-size analysis was kindly provided by Pfeiffer (Radolfzell, Germany). All other reagents used were of pharmaceutical grade.

Experimental Design

A 5-factor (HU, HEC, PEO, NaCl, and $CaCl_2$), 3-level Box-Behnken design on the measured responses (rheology, droplet size, and in vitro drug release) was established for this optimization procedure. The nonlinear quadratic model generated by regression of the variables is as follows:

$$Y = b_0 + b_1 * HEC + b_2 * PEO + b_3 * CaCl_2 + b_4 * NaCl + b_5 * HU + b_6 * HEC * HEC + b_7 * PEO * PEO + b_8 * CaCl_2 * CaCl_2 + b_9 * NaCl * NaCl + b_{10} * HU * HU + b_{11} * HEC * PEO + B_{12} * HEC * CaCl_2 + b_{13} * HEC * NaCl + b_{14} * HEC * HU + b_{15} * PEO * CaCl_2 + b_{16} * PEO * NaCl + b_{17} * PEO * HU + b_{18} * CaCl_2 * NaCl + b_1 + b_{19}CaCl_2 * HU + b_{20} * NaCl * HU + E,$$

where Y is the measured response associated with each factor level combination; HEC, PEO, $CaCl_2$, NaCl, and HU are the factors studied; b_0 to b_{20} are the regression coefficients; and E represents the error term.[7] The independent factors and the dependent variables used in the design are listed in Table 1. This study design, requiring a total of 44 experimental runs (formulation combinations), was generated and analyzed using MINITAB 14.

Rheological Characterization of Test Formulations

The rheological behavior of the test formulations was investigated using a small sample adapter attached to the LV-DV-III Brookfield viscometer (Brookfield, Middleboro, MA). Rheological profiles were performed by linearly increasing the shear rate (13.20 to 132.00 seconds^{-1}) followed by a stepped reduction in shear rates. Rheological constants were obtained by regression using Rheocal software (Version 2.3, Brookfield).

In-Vitro Drug Release Studies

In-vitro drug release studies were performed using Franz diffusion cells (Hanson Research, Chatsworth, CA). Dialysis membranes (6000-8000 Dalton molecular weight cut-off) were mounted between the receiver and donor compartments of the diffusion cells maintained at 37°C. Test formulation (200 μL) was placed in the donor compartment, and the receptor compartment was filled with deionized water (5 mL). The contents were stirred continuously at a controlled speed with a magnetic stirrer (400 rpm). At predetermined times, 1-mL samples were withdrawn from the receptor compartment and replenished with an equal volume of deionized water. All in vitro drug release studies were performed in triplicate, and

Table 1. The Variables Used in the 5-factor, 3-level Box-Behnken Design Using MINITAB 14 Software*

Independent Variables	Levels		
	Low	Middle	High
HEC (%)	0	2	4
PEO (%)	0	2	4
$CaCl_2$ (%)	0	15	30
NaCl (%)	0	15	30
HU (%)	0	2	4
Dependent variables	**Low**	**High**	**Objective**
Viscosity (cP)	1	110	Minimize
In vitro drug release (MDT)	0	3.6	Maximize
Droplet size-Dv_{50} (μm)	56	192	Minimize

*HEC indicates hydroxyethylcellulose; PEO, polyethylene oxide; $CaCl_2$, calcium chloride; NaCl, sodium chloride; HU, hydroxyurea; and MDT, mean diffusion time.

samples were assayed for HU using high-performance liquid chromatography (HPLC). The mean dissolution times (MDT) were calculated to represent drug release using the following equation[8,9]:

$$MDT = \sum_{i=1}^{n} \tau i(Mt/M\infty) \quad (1)$$

Where M is the fraction of dose released in time $\tau i = (t_i + t_{i-1})/2$ and $M\infty$ corresponds to the loading dose. Thus, MDT can be referred to as "mean diffusion time."

HPLC Assay for HU

A Waters HPLC system with 600E pump and 717 plus autosampler was used (Waters Corp, Milford, MA). Electrochemical detection of HU was performed with an ESA Coulochem II amperometric detector (ESA Biosciences Inc, Chelmsford, MA). Isocratic separation was achieved at 27°C using an YMC column (150 mm × 3 mm; Waters). The working electrode was set at an applied potential of 700 mV relative to an Ag/AgCl reference electrode; filter setting was 0.1 Hz; and range setting was 10 nA. The mobile phase consisted of 7.2 mM citric acid and 11 mM sodium dihydrogen phosphate as supporting electrolyte in 85/15 water/acetonitrile composition. Each run required 10 minutes (peak time, 1.8 minutes) at a flow rate of 0.5 mL/min.

Determination of Droplet Size Distribution From Test Formulations

The experimental method is described in more detail in a previous publication.[1] In brief, droplet size analysis of nasal aerosols was conducted by laser diffraction using a Malvern Spraytec with RT Sizer software (Malvern Instruments Ltd, Worcestershire, UK). InnovaSystems nasal actuation station (Moorestown, NJ) with "Might Runt" software was used to actuate the nasal pumps.

DSD measurements were conducted at 3 cm from the laser beam. All measurements were made at room temperature (21°C-23°C). Data were reported as volume diameter defined by 10%, 50% (volume median), and 90% of the cumulative volume undersize (Dv_{10}, Dv_{50}, and Dv_{90}, respectively).

Measurement of Dynamic Surface Tension

Surface tension measurements were performed using the SITA T60/2 tensiometer (SITA Messtechnik, Dresden, Germany), which employs the maximum bubble pressure method. DST measurements were conducted at room temperature (23°C) at bubble lifetimes in the range 0.03 to 60 seconds per bubble (giving corresponding surface ages for surfactant-like molecules to adsorb to the liquid-air interface). The instrument was calibrated using water, and a surface reading of 72.8 ± 0.1 mN/m was regarded as accurately standardized.

RESULTS

Effect of Formulation Components on Viscosity

The viscosity of PEO and HEC formulations exhibited mild shear-thinning behavior (Figure 1). However, the viscosity of formulations of both the polymers was significantly affected with the addition of electrolytes. The coefficients for the polynomial equation relating the response and independent variables are shown in Table 2.

The values of the coefficients for PEO, HEC, NaCl, $CaCl_2$, and HU relate to the effects on the viscosity. Coefficients (Coef, Table 2) with more than one factor term represent the interaction terms and coefficients with higher terms (SE Coef, Table 2) indicate the quadratic (nonlinear) nature of the relationship. A positive sign indicates a synergistic effect, while a negative sign represents an antagonistic effect. The theoretical (predicted) values and the observed (experimental) values were in excellent agreement with a correlation of $r^2 = 0.986$ as shown in Table 2. The coefficients reflect PEO and HEC influenced the viscosity, whereas $CaCl_2$ and NaCl showed interactions with PEO and HEC that resulted in changes in viscosity. HU showed the least influence on the viscosity. The surface plots in Figure 2 show the effect of electrolytes on the viscosity of PEO and HEC gels. From the figure it is evident that the addition of NaCl to PEO reduces the viscosity of the solution. For example, the viscosity of a 4% wt/vol PEO solution is reduced by 33% with the addition of 30% wt/vol NaCl. However, the addition of $CaCl_2$ to PEO had an opposite effect on the viscosity. There was a 25% increase

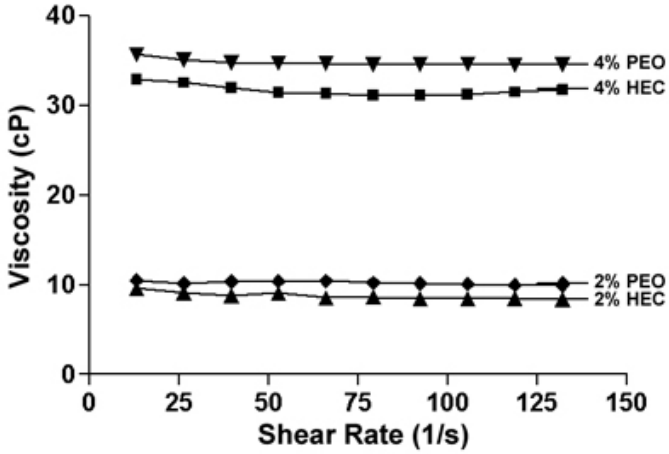

Figure 1. Shear viscosity of solutions of PEO and HEC at various concentrations.

Table 2. Quadratic Model and the Coefficients for the Viscosity, In Vitro Drug Release, and Droplet Size (Dv_{50}) From Formulations of HU*

No. of Variables: 5			
	Viscosity	In Vitro Drug Release	Droplet Size
R^2	98.6%	93.5%	86.2%
Regression Coefficients			
Term	Viscosity Coef, SE Coef	In Vitro Drug Release Coef, SE Coef	Droplet Size Coef, SE Coef
Constant	10.2337, 7.0160	20.1038, 31.2080	−0.1556, 0.8807
HEC	−7.0546, 2.3644	35.3040, 10.5171	0.4201, 0.2968
PEO	−3.5226, 2.3644	65.3821, 10.5171	−0.0356, 0.2968
DRUG	−2.6711, 2.3644	−9.1356, 10.5171	2.9257, 0.2968
NaCl	0.1284, 0.3153	1.8093, 1.4023	−0.0075, 0.0396
CaCl2	−0.4676, 0.3153	4.3340, 1.4023	−0.0011, 0.040
HEC*HEC	3.1699, 0.3435	−1.6799, 1.5278	−0.0618, 0.0431
PEO*PEO	1.9489, 0.3435	−7.3861, 1.5278	−0.0395, 0.0431
DRUG*DRUG	0.3330, 0.3435	0.4909, 1.5278	−0.6356, 0.0431
NaCl*NaCl	0.0064, 0.0061	0.0100, 0.0272	−0.0004, 0.0008
CaCl2*CaCl2	0.0091, 0.0061	−0.0483, 0.0272	0.0003, 0.0008
HEC*PEO	4.1306, 0.4667	−4.7250, 2.0759	−0.0056, 0.0586
HEC*DRUG	0.4869, 0.4667	−0.6312, 2.0759	−0.0325, 0.0586
HEC*NaCl	−0.0612, 0.0622	−0.0200, 0.2768	0.0102, 0.0078
HEC*CaCl2	−0.0106, 0.0622	−0.8533, 0.2768	−0.0125, 0.0078
PEO*DRUG	−0.2050, 0.4667	3.0687, 2.0759	0.0088, 0.0586
PEO*NaCl	−0.2280, 0.0622	−0.7267, 0.2768	0.0024, 0.0078
PEO*CaCl2	0.1357, 0.0622	0.2775, 0.2768	0.0088, 0.0078
DRUG*NaCl	0.0192, 0.0622	−0.0183, 0.2768	0.0020, 0.0078
DRUG*CaCl2	0.0583, 0.0622	0.0642, 0.2768	−0.0023, 0.0078
NaCl*CaCl2	0.0037, 0.0083	−0.0071, 0.0369	−0.0008, 0.0010

*Abbreviations are explained in the first footnote to Table 1.

in viscosity by the addition of 30% wt/vol $CaCl_2$ to PEO. There were significant but less dramatic alterations in the viscosity with the addition of electrolytes to HEC. While NaCl had a minor effect in reducing the viscosity of HEC formulations, $CaCl_2$ significantly increased the viscosity of HEC formulations. Addition of HU at 1% to 4% wt/vol concentration to PEO produced no apparent change in viscosity or showed only a minor effect in increasing viscosity of HEC formulations (data not shown). Combinations of the 2 polymers, HEC and PEO, demonstrated a synergistic effect on viscosity as shown in Figure 2. The concentration origins for PEO (x-axis) and HEC (z-axis) start at opposite ends, indicating that the 2 polymers competed for solvency resulting in a higher viscosity.

Effects of Formulation Components on the In Vitro Drug Release of HU

A high value for MDT is indicative of retarded drug release, whereas a lower value is indicative of accelerated release. The predicted responses versus actual data from the various formulations are summarized in Table 2. The theoretical (predicted) values and the observed (experimental) values were in good agreement with a correlation of r^2 = 0.935. Figure 3 shows the effect of various formulation components on the release of HU from the polymer gel vehicles. As shown in Figure 3, HU release exhibited a parabolic relationship with both HEC and PEO polymers with peak retardation in the drug release (as shown by increased MDT values) at 2.5% wt/vol HU concentration. This finding suggests that there is an attraction or complexation of HU with the polymers. In addition, as shown in surface plots of PEO and HEC, even in the presence of polymers together, the parabolic relationship in the drug release existed, as evidenced by the umbrella configuration of the plot. The addition of $CaCl_2$ to PEO further retards the release of HU. A parabolic effect on HU release from PEO was observed with the addition of NaCl as shown in Figure 3. This surface plot exhibits an umbrella configuration signifying that NaCl accelerated the release of HU at low concentrations (below 10% wt/vol NaCl) and at higher concentrations (25%-30% wt/vol NaCl) from PEO. The influence of $CaCl_2$ on the extent of HU release in the presence of HEC is shown in Figure 3, where there is a downward curve in MDT with increasing levels of $CaCl_2$. This increase in HU release is contrary to the contribution

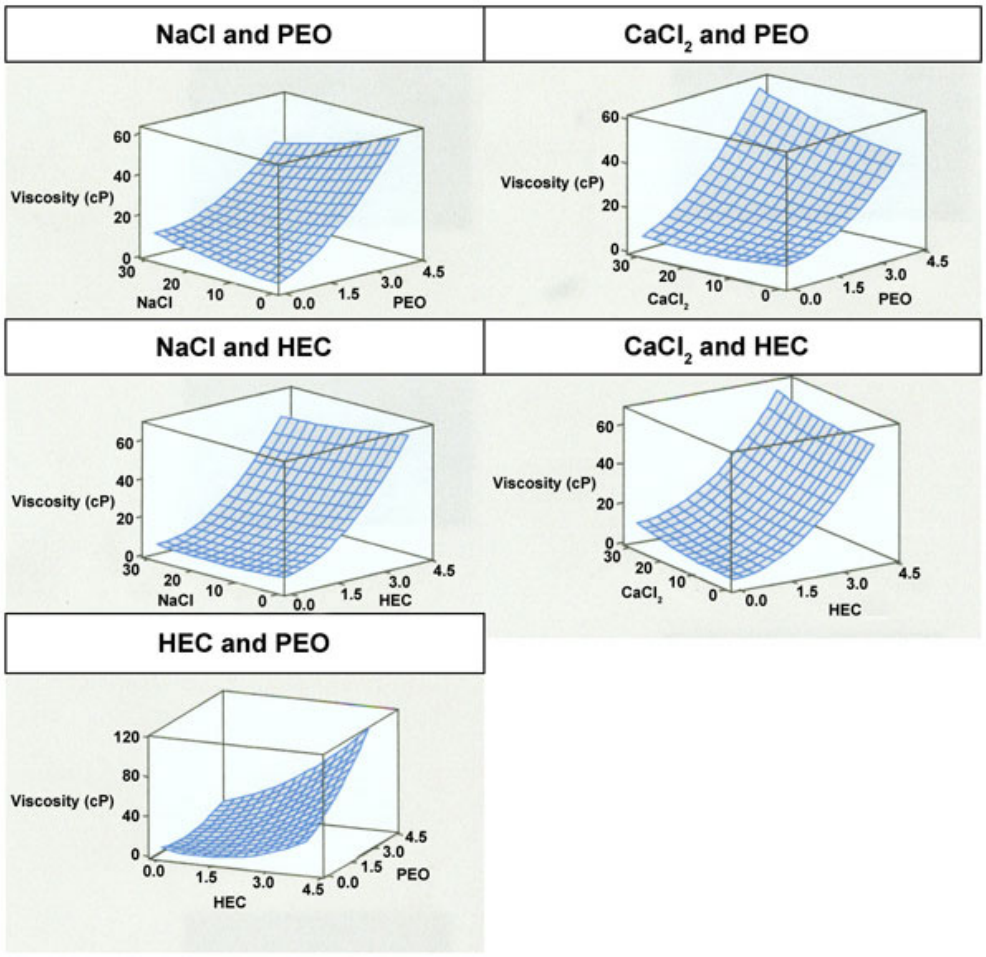

Figure 2. Response surface plot (3D) showing the effect of various formulation components on the viscosity.

to increased viscosity suggesting that Ca^{2+} ions are competitively displacing HU from HEC. Also the action of NaCl accelerates HU release from HEC as a function of concentration (data not shown).

Effects of Formulation Ingredients on Droplet Size

Surface response plots were generated from the median droplet size (Dv_{50}) for 44 designed formulations as shown in Figure 4. The predicted responses versus actual data are summarized in Table 2. The lower than expected correlation was attributed to other variables affecting droplet formation (discussed later) and experimental error. Surface plots are presented to confirm and elucidate the effect of excipient interactions on the droplet size. Both PEO and HEC increased the Dv_{50} in a concentration dependent manner with PEO contributing to larger Dv_{50} values as opposed to HEC. The addition of electrolytes to HEC and PEO also significantly influenced the droplet size. For example, the addition of NaCl to PEO resulted in lower Dv_{50}. However, the addition of $CaCl_2$ to PEO demonstrated a mild parabolic relationship, with a reduction in Dv_{50} values at low concentrations (10%-15% wt/vol) of $CaCl_2$. The addition of HU to PEO resulted in higher Dv_{50} values, whereas the opposite effect occurred with HEC. The Dv_{50} of HEC was increased by the addition of NaCl but exhibited a mild parabolic relationship with $CaCl_2$.

To examine the true implication of these interactions on the DSD, additional droplet size experiments were conducted. Subsequently, the effects of polarity/ionic strength on the DSD as well as electrolyte-polymer effects were studied. DSD plots in Figure 5 show that electrolytes altered the DSD compared with water. The DSD profile from 0.5% $CaCl_2$ solution demonstrated significantly lower Dv_{50} value compared with water ($F_{5,48}$ = 136.5, P < .001). As the concentration of $CaCl_2$ was increased above 10% wt/vol, the Dv_{50} was statistically higher than water in a progressive manner. On the other hand, NaCl concentration up to 20% wt/vol demonstrated significantly lower Dv_{50} values as compared with water. Only at 30% wt/vol concentration, NaCl exhibited statistically similar Dv_{50} value to water. The rank order of droplet size (Dv_{50}) is 1% NaCl > 10% NaCl > 15% NaCl > 20% NaCl > water = 30% wt/vol NaCl. Figure 5 also shows that the addition of $CaCl_2$ to a 2% PEO solution in the range of 10% to 15% shifted the

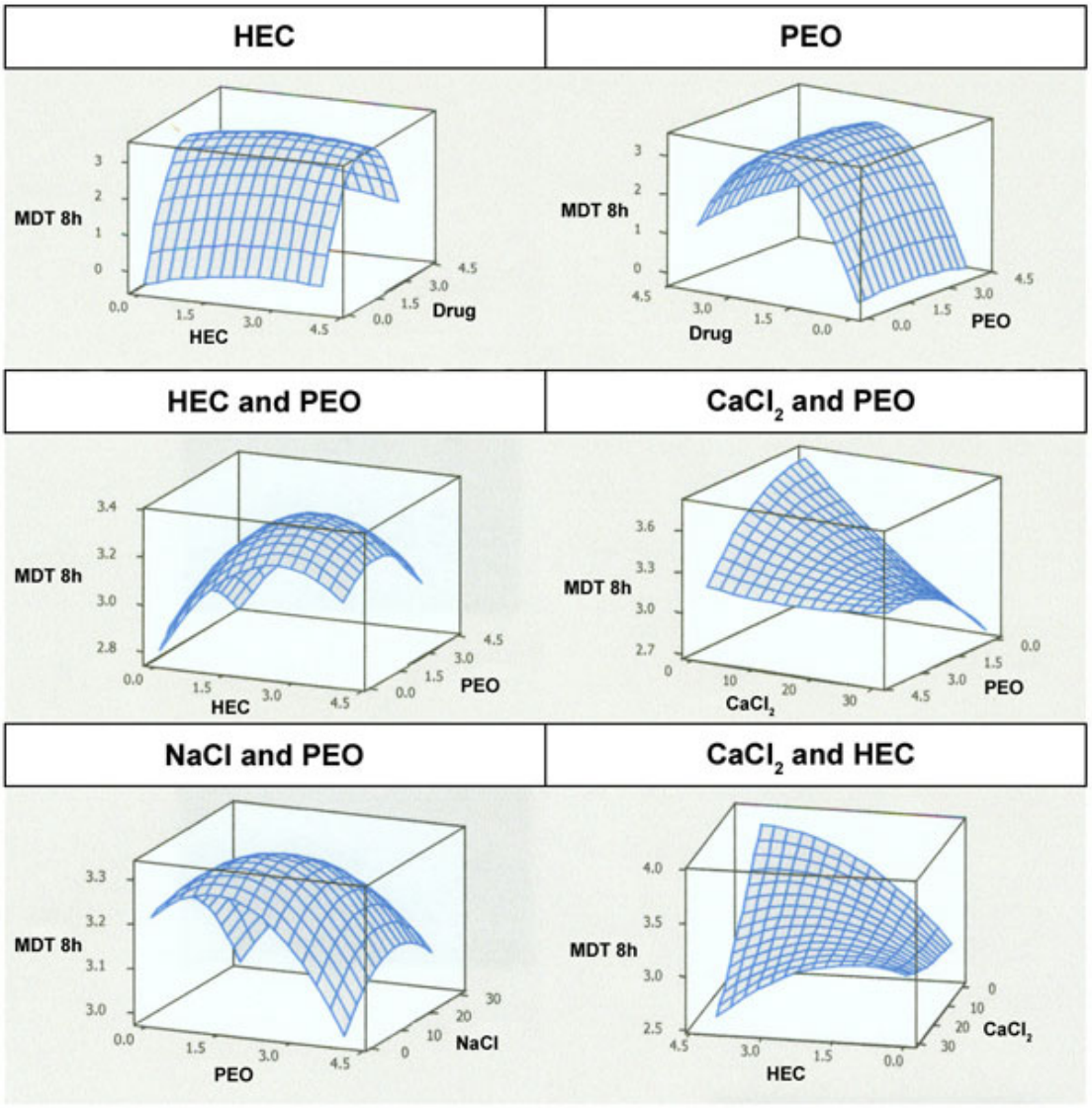

Figure 3. Response surface plot (3D) showing the effect of formulation components on the MDT of HU.

DSD curve toward smaller droplet size compared with 2% PEO. Conversely, $CaCl_2$ concentration between 20% and 30% wt/vol increased the DSD toward higher droplet size. Similar parabolic relationship in DSD was also exhibited with the addition of NaCl to PEO with a peak reduction in DSD at 15% NaCl concentration (a reduction of 60% compared with PEO). However, any benefit in DSD reduction was virtually eliminated when the concentration of NaCl was increased to 30%.

HEC also exhibited significant changes in DSD with the addition of electrolytes. However, these effects were not as dramatic compared with PEO. We observed a parabolic relationship with HEC and NaCl on the DSD with a peak reduction in DSD at 10% wt/vol NaCl (16% compared with HEC alone). NaCl at a concentration above 10% wt/vol increased DSD toward larger droplet size, and at 30% wt/vol NaCl exhibited a higher DSD, which is 25% greater than HEC alone. In the case of $CaCl_2$, up to 15% concentration, the Dv_{50} value was the lowest. Subsequent increases of $CaCl_2$ resulted in steady increase in Dv_{50} values.

Surface Tension

The effect of various concentrations of polymers and electrolytes on DST is shown in Figure 6. The surface tension versus time plots for HEC and PEO indicate that these polymers exhibit surface-active properties. Increasing the concentrations of HEC and PEO from 0.5% to 4% wt/vol resulted in a higher surface tension at low surface ages (30-1000 milliseconds) followed by a reduction in surface values until approximately the same surface tension values were reached. (Corresponds to 65 mN/m and 60 mN/m for HEC and PEO, respectively). From these results no significant characteristic difference in the general behavior between HEC and PEO was detected apart from the fact that PEO showed a shorter induction time and lower surface tension, which indicates a difference in adsorption dynamics.

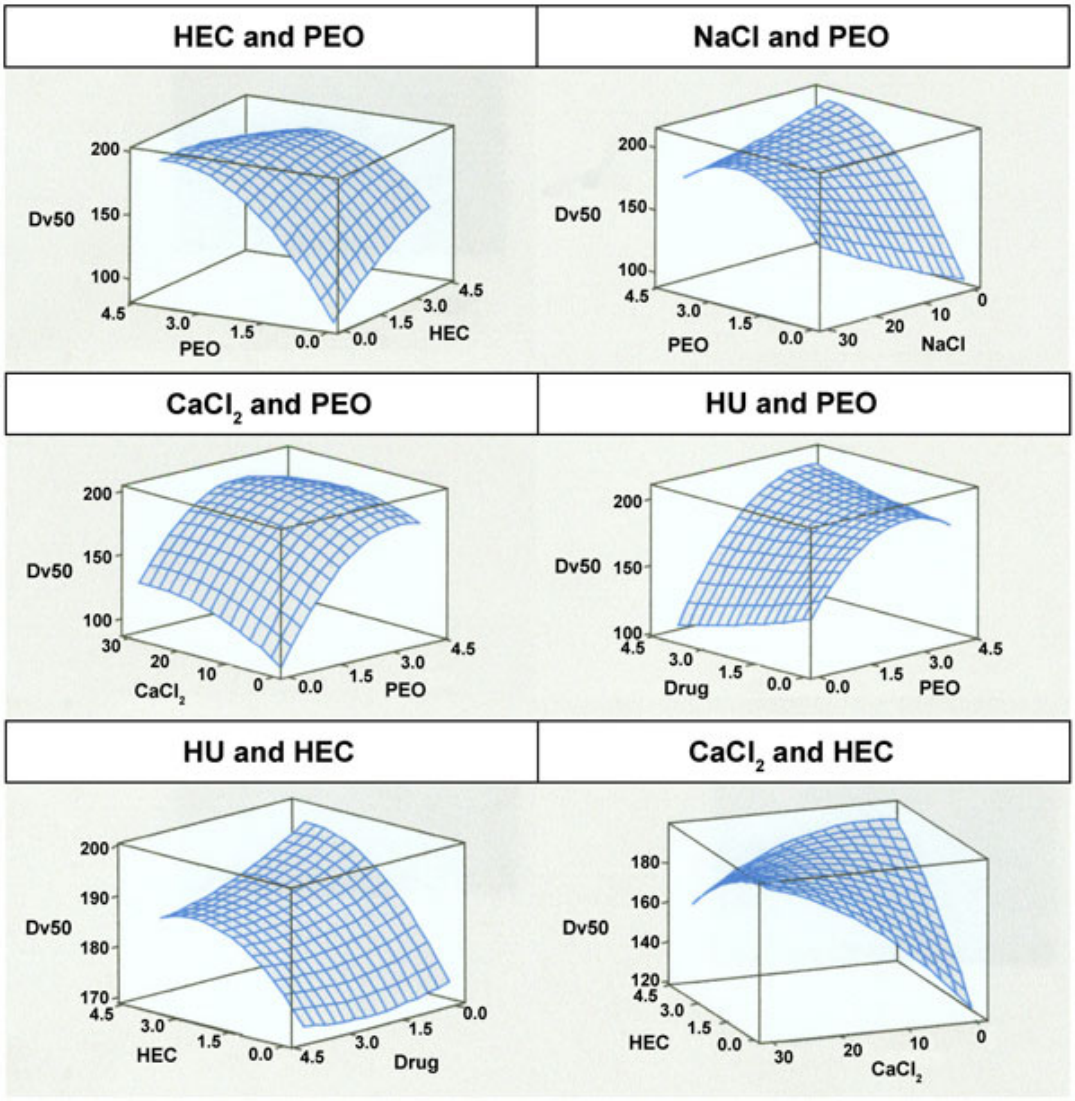

Figure 4. Response surface plot (3D) showing the effect of formulation components on Dv_{50}.

The influence of electrolytes on the DST is also shown in Figure 6. Increasing the ionic strength of the solution by the addition of $CaCl_2$ and NaCl led to an increase in surface tension in a concentration-dependent manner. However, at concentration of 30% wt/vol, both electrolytes exhibited lower surface tension initially followed by a sharp increase with time. This phenomenon occurs below surface age of 200 milliseconds for both electrolytes. The effect of NaCl and $CaCl_2$ on DST of both PEO and HEC gels is shown in Figure 7. This figure shows that at various concentrations of NaCl, the surface properties of HEC were only slightly affected. Addition of $CaCl_2$ did not significantly alter HEC surface behavior except at a 30% wt/vol concentration. The addition of NaCl to PEO solution led to the reduction of surface tension at all surface ages in a concentration-dependent manner suggesting that the surface of the solution was becoming more hydrophobic. However, the addition of $CaCl_2$ at various concentrations did not alter the DST profiles of PEO.

DISCUSSION

Influence of Formulation Components on Viscosity

Viscosity Changes of HEC Due to Electrolytes

The addition of HU and electrolytes to HEC or PEO altered the flow properties of the solution. These changes are presumably due to alterations in polymer chain conformations (ie, chain to chain conformations and chain flexibility). The interaction of electrolytes with polymers can also be explained by the phenomenon of aggregation of the polymer units arising from hydrogen bonding and hydrophobic interactions.[10-12] The addition of NaCl to HEC appeared to have little effect on the viscosity and aggregation process, except at high polymer concentrations, where the presence of electrolyte led to a slight reduction in viscosity. This result can be explained by the preferential attraction of electrolyte ions to an aqueous environment, resulting in the solvent having a higher effective polarity.[13-15] This reduces the polymer-polymer aggregating interactions and increases

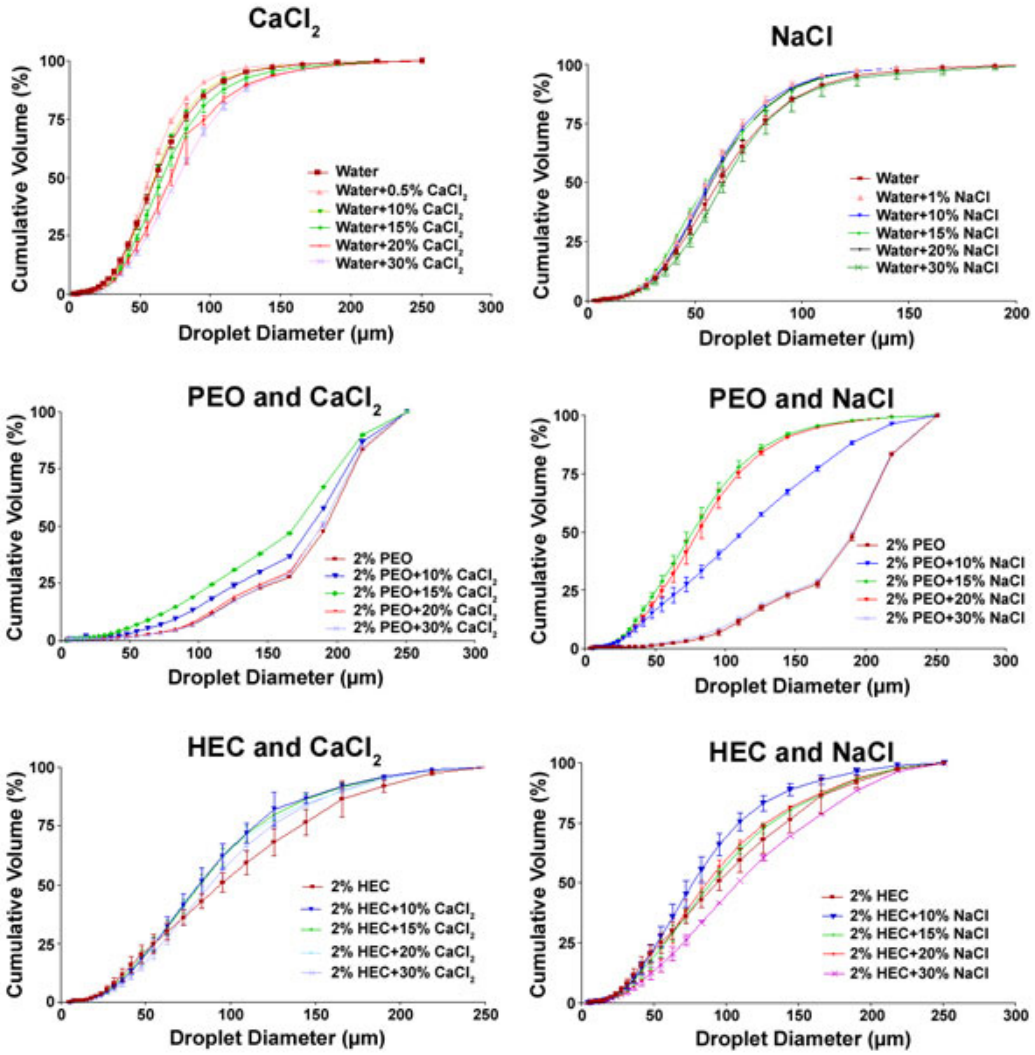

Figure 5. Effect of salts concentration on the DSD from HEC, PEO gels. Each data point represents the average ± SD of 6 actuations. For each graph, statistical analysis was performed using 1-way analysis of variance (ANOVA) on Dv_{50} values with Tukey's multiple comparison test. All show significant differences except ($P > .05$): 10% $CaCl_2$ vs water; 1% NaCl vs 10% NaCl; 1% NaCl vs 15% NaCl; 1% NaCl vs 20% NaCl; 10% NaCl vs 15% NaCl; 10% NaCl vs 20% NaCl; 15% NaCl vs 20% NaCl; 30% NaCl vs water; 2% PEO + 20% $CaCl_2$ vs 2% PEO + 30% $CaCl_2$; 2% PEO vs 2% PEO + 30% NaCl; 2% HEC vs 2% PEO + 30% $CaCl_2$; 2% HEC + 10% $CaCl_2$ vs 2% HEC + 15% $CaCl_2$; 2% HEC + 10% $CaCl_2$ vs 2% HEC + 20% $CaCl_2$; 2% HEC + 10% $CaCl_2$ vs 2% HEC + 30% $CaCl_2$; 2% HEC + 15% $CaCl_2$ vs 2% HEC + 20% $CaCl_2$; 2% HEC + 15% $CaCl_2$ vs 2% HEC + 30% $CaCl_2$; 2% HEC + 20% $CaCl_2$ vs 2% HEC + 30% $CaCl_2$.

the hydrophobicity of the polymer.[10-12] Surface tension data in Figure 7(HEC and NaCl) provide some support for this premise. This figure shows that NaCl increases the initial surface tension followed by equilibration to a steady-state. This initial rise in surface tension is due to the effect of NaCl on the polarity of the solution, whereas the lower surface tension was the result of HEC forming hydrophobic clusters. The addition of $CaCl_2$ to HEC appeared to have little effect on the viscosity and aggregation process, except at high polymer concentrations, where the presence of electrolyte caused a slight increase in viscosity. Ca^{2+} ions, due to their large charge density interactions with water molecules, form a hydrated ion shell. This shell restricts and reduces solvent diffusion and thus manifests in resisting polymer movement and increased viscosity.[16-18]

Viscosity Changes in PEO Due to Electrolytes

The ionic excipients produced a dramatic effect on viscosity of PEO solutions; NaCl produced maximum reduction in the viscosity of PEO solutions, as shown Figure 2. This reduction is attributed to the PEO polymer coiling around the Na^+ ions. The polymer forms circular coils that condense into individual clusters, which reduce the polymer-polymer interactions, and in turn reduce flow resistance. This premise is also supported by DST data as

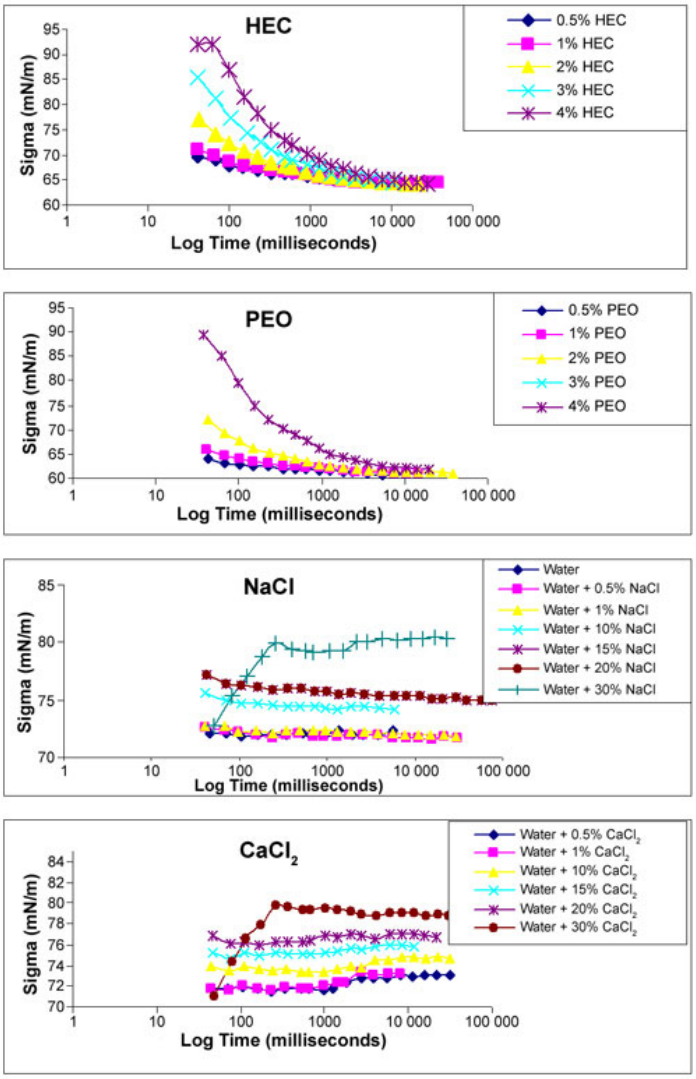

Figure 6. DST changes at various concentrations of PEO, HEC, NaCl, and $CaCl_2$.

shown in Figure 7. The reduction in viscosity and DST of PEO by NaCl is postulated as follows: the flexible oxygen group of the polymer chain forms an electrostatic attraction for Na^+ ions. The opposing $-CH_2-$ groups of the polymer chain are repelled and the polymer coils due to hydrophobic attractions of the various $-CH_2-$ groups. Thus, the polymer surface area in the bulk solution is decreased with the oxygen groups exposed interacting with the Na^+ ions. Based on reductions in viscosity (Figure 2), droplet size (Figure 5), and DST (Figure 7), consequently from the addition of NaCl to PEO, a proposed mechanism for the formation of PEO/NaCl complexes is depicted schematically in Figure 8. The model proposed is based upon the concept that the electronegative nature of the PEO chain allows it to coordinate with Na^+ ions to form a "pseudo-polycation." At a specific NaCl concentration Na^+ ion aggregates are formed and the concentration of free Na^+ ions increases substantially. These free cations can then shield the electrostatic repulsion between the PEO segments, thereby causing a partial contraction of the coils and a reduction in viscosity.[10-12,17,18] Hydrophobic attractions are strengthened by $-CH_2-$ groups flanked on either side of the oxygen groups that are able to interact with other $-CH_2-$ groups within or adjacent to other polymer chains.[17,18]

The addition of $CaCl_2$ to PEO increases the viscosity, and this is mainly due to electrolyte interaction with water. This interaction of electrolytes with water is important because there are some anomalous circumstances in relating $CaCl_2$ interaction with PEO. There were disparate actions such as

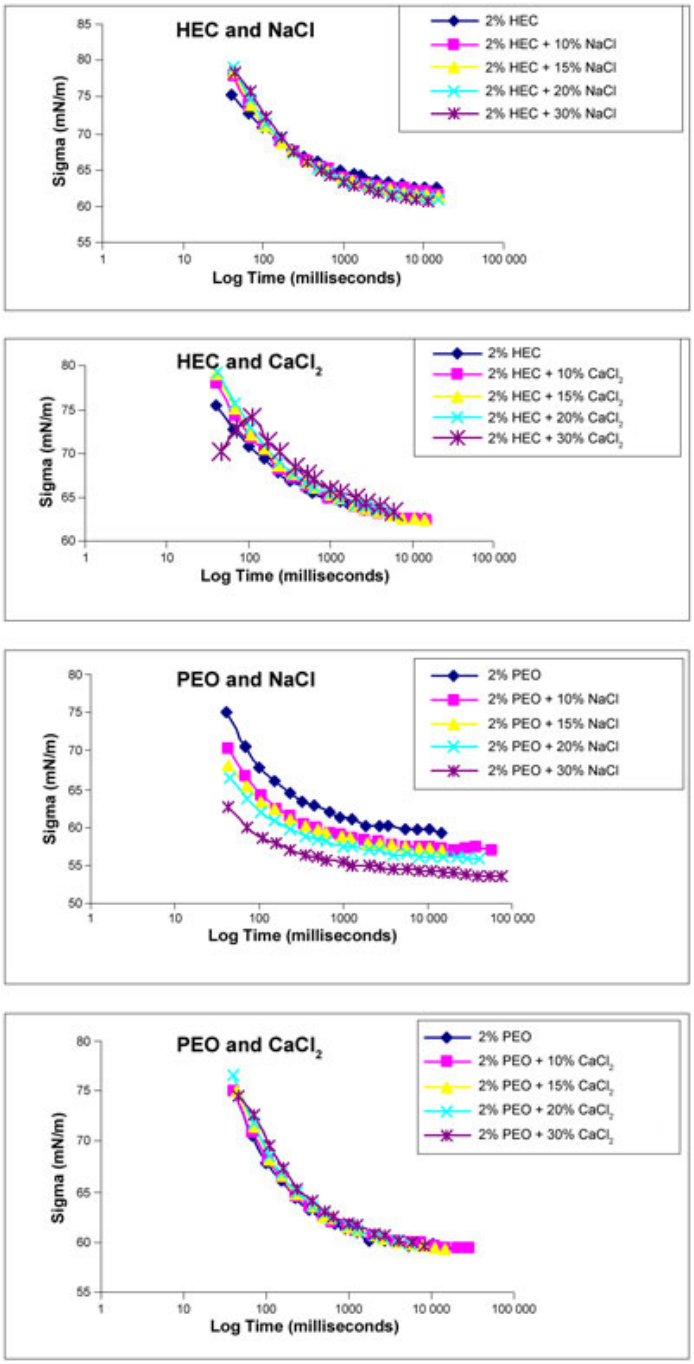

Figure 7. DST changes arising from the addition of electrolytes to either a 2% HEC or 2% PEO gels.

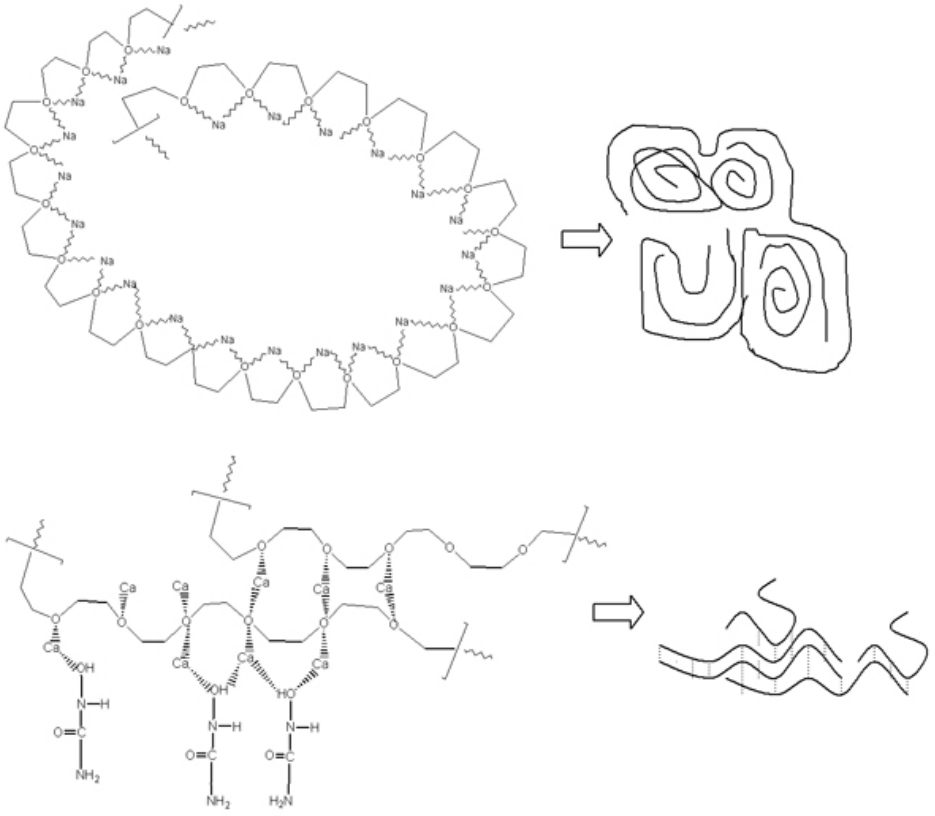

Figure 8. The proposed changes in rheology of PEO with Na^+ ions to form a "pseudopolycation." The figure shows the proposed changes in rheology and MDT from PEO gel with HU and Ca^{2+}.

increased viscosity (Figure 2), lower droplet size (Figure 5), and no changes in DST behavior (Figure 7) as well as increase in HU release (Figure 3).

Effects of Formulation Ingredients on the In Vitro Release of HU

MDT represents the cumulative percentage (%) fraction released for each time interval. An elevated value for MDT is indicative of a smaller fraction of drug released. The dome-shaped plots in Figure 3 indicate that both HEC and PEO polymers form a weak complex with HU, similar to ligand binding. In these figures there is a linear increase in MDT as seen on the z-axis; on the y-axis there is an increase up to a certain extent, followed by a reduction. This indicates that a critical HU concentration is involved up to which retardation of drug release is favored. The process of complexation rather than viscosity significantly controls release of HU. Around the critical concentration (2.5% HU), the competing polymer-polymer interactions and changes in solvency reduce the number of sites for the HU to form complex, thereby resulting in a reduction in the MDT. This behavior is also shown in Figure 3, which shows an umbrella-shaped plot of the competing actions of PEO and HEC for HU-polymer complex. The parabolic effect in the MDT for HU with the addition of NaCl to PEO can be explained by direct and indirect mechanisms. The sequence of events starts with Na^+ ions at a low concentration, which disrupts the hydrogen bonding between HU and PEO. HU is competitively displaced by Na^+ ions. Additional Na^+ ions unhinge the symmetry of the oxygen segments of the polymer chain resulting in polymer coiling and condensing. This coiling creates exterior and interior compartments[10-12,17,18] providing a temporary barrier that prevents competition of HU and Na^+ for sites on the polymer. As more Na^+ ions are added, further polymer condensation occurs and the viscosity of the solution is decreased. This reduction in viscosity along with the displacement of HU into the bulk of the solution accelerates the release of HU.

The addition of $CaCl_2$ to PEO retards HU release as shown in Figure 3. The response plots show an upward curvature in the MDT with increasing $CaCl_2$, which suggests that more than one factor influences HU release. Ca^{2+} ions do not appear to compete or displace HU-PEO complexes. As there were no changes in surface the tension behavior, 2 plausible events could explain HU retardation with the addition of $CaCl_2$. Ca^{2+} ions can directly bind with several HU molecules via the electronegative oxygen groups. These large shells of water and HU molecules surrounding the Ca^{2+} ion can act independently or alternatively

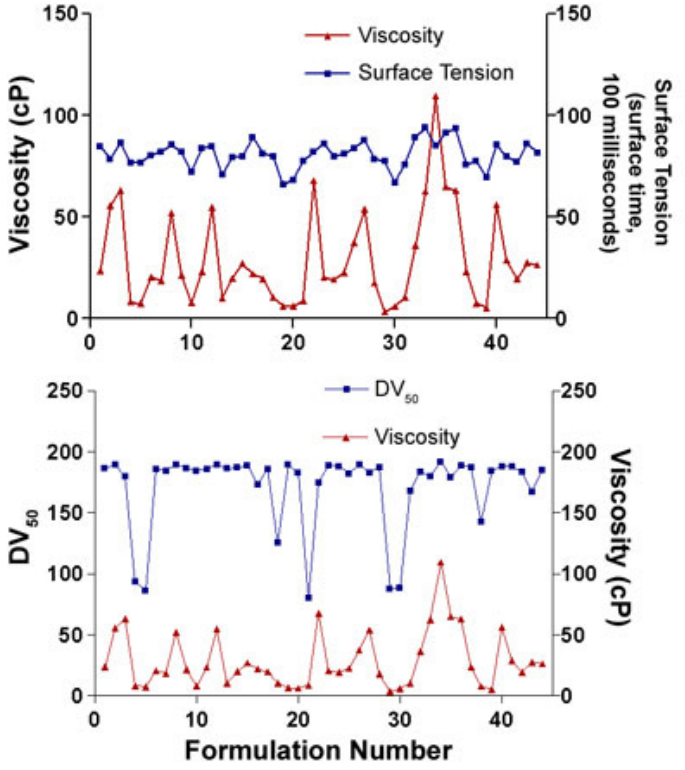

Figure 9. Relationship between viscosity and surface tension vs Dv_{50} and MDT. Dv_{50} vs viscosity $r^2 = 0.17$; Dv_{50} vs DST $r^2 = 0.09$; Viscosity vs DST $r^2 = 0.45$; MDT vs viscosity $r^2 = 0.01$.

associate with PEO. Experimental data support this association, and Ca^{2+} ions act as bridging molecules between HU and PEO and thus retarded the release of HU. This premise is depicted in Figure 8. The polymer structure is not significantly altered but becomes heavier (increases in network density).[13] In conjunction with the bulky shell groups, Ca^{2+} ion high density charge restrict diffusional movement. The enhanced complexation and increased viscosity both aid in slowing the release of HU.

As shown in Figure 3, there is a downward curve in MDT with increasing levels of $CaCl_2$ to PEO. This increase in HU release is contrary to the contribution to increased viscosity suggesting that Ca^{2+} ions are competitively displacing HU from HEC. The addition of NaCl to HEC decreased MDT in a concentration-dependent manner (data not shown). The displacement of HU from HEC with the addition of NaCl is also augmented by a very small reduction in viscosity.

Influence of Formulation on Droplet Size

Role of Viscosity and DST on Droplet Size

The influence of surface tension and viscosity on droplet size has been reported using different medical devices. Some reports have demonstrated correlation of either surface tension or viscosity to the droplet size as generated from simple solutions.[19-21] A typical plot of surface tension versus time of a surfactant solution follows a sigmoid pattern.[22] From this pattern it is possible to identify separate consecutive kinetic regions: the induction period, the surface coverage, and finally a progressive ordering of surfactant segments within the surface layer. The lower than the expected correlation for droplet size using DOE was attributed to the critical actions of other variables that altered DSD. These variables include changes in the solvent properties, density of solution, and competing actions of aerodynamic properties (surface tension and viscosity). To illustrate the impact of these properties, DSD plots were generated for both electrolytes and polymers (Figure 5). In addition, a series of linear correlation analysis was performed to determine whether viscosity or surface tension (at various surface ages) had a direct influence on the Dv_{50} from the 44 formulations as illustrated in Figure 9. The lack of any significant linear correlation between physicochemical parameters with droplet size or with in vitro drug release as reported in Figure 9 was a consequence of many factors such as formulation interaction, chemistry, and complex interplay of aerodynamic forces. As shown in Figure 9, a general trend is observed in which a decrease in viscosity or surface tension results in a reduction in Dv_{50} for certain formulations but not for others. In addition, beyond a certain viscosity range, the linear relationship between Dv_{50} and viscosity failed as a result of changes in the aerodynamic forces. The aerodynamic forces include surface (cohesive) and viscous (frictional) forces relative to their resistance to break up into droplets due to external pressure and liquid-air turbulent behavior. As such changes in these surface and viscous forces complicate the relationship for predicting droplet size. Several investigators have attempted, using mechanical sprays, to predict DSD from simple solutions based on empirical means.[23,24]

The focus of this study has been on some of the formulation influences as well as nonionic/ionic interactions and their effect on DSD. From DST profiles (Figures 6 and 7), the individual components produce a characteristic profile that is dependent on the concentration of each component. The electrolytes did not create major differences in the DST profile of polymers with the exception of their high concentration (30% wt/vol), where they demonstrated lower DST (< 200 milliseconds). The wide viscosity differences can be attributed to the type of polymer and its concentration and also to nonionic/ionic interactions as discussed earlier. The exact relationship between droplet Dv_{50} from 44 formulations and physicochemical properties is too complex to relate to the viscosity and surface tension in isolation. Conflicting experimental findings on the above relationships on the atomization contributed to difficulties in predicting the relationships using DOE.[25-27] For example,

there is a decrease in Dv_{50} with an increase in dynamic surface tension using electrolyte solutions. This finding suggests that other factors such as density or solvent properties also played a significant role. From Figure 5 it is obvious that density had a minor influence since increasing the concentration of electrolytes did not correspond to the Dv_{50} in an incremental fashion.

Changes in solvent properties may explain the anomalous lower DSD values with low concentration of electrolytes and higher surface tension compared with water (Figures 5 and 6), which is a contradictory effect that cannot be attributed to viscosity or surface tension. Thus, proposed are the effects of polar interactions on the hydrogen bonding of water that can explain the observed changes in DSD.

The Effect of Electrolytes on Solvent Properties

It has been hypothesized that water exists in loose conformations arising from hydrogen bonding within itself. If a polar solute molecule is placed in water then the positive ends of the solvent molecules will attract the negative ends of the solute molecules. This type of intermolecular force is known as dipole-dipole interaction. The electrolytes in water exist as loosely associated ion pairs that are each solvated by water (between 6 and 8 molecules) but are still strongly attracted to one another and tend to move in pairs, in concert with one another. Both the extent and strength of hydrogen bonding may be changed independently by the solute. The effects and extent of the quality of hydrogen bonding is of overriding importance.[28,29]

Large singly charged ions, such as Na^+, with low charge density exhibit weaker interactions with water than water with itself and thus, interfere little in the hydrogen bonding of the surrounding water molecules. Whereas, multiple-charged ions, with high charge density, such as Ca^{2+}, exhibit stronger interactions with water molecules than water with itself and therefore are capable of breaking hydrogen bonds between water molecules.[19-21] These interactions result in hydrogen bonds possessing reduced strength in the bulk of the solution and thereby can be atomized into smaller droplets. However, at high electrolyte concentrations, there is an increase in DSD. High amounts of electrolytes will increase the density of the solution and may result in decreased self-diffusion as the molecules restrict each other's movements. The self-diffusion of water molecules becomes restricted especially more so with Ca^{2+} due to its large charge density and hydrated ion shell. Electrolyte ions prefer to be fully hydrated in the bulk liquid water and so increase the surface tension by adding to the attractive forces on the surface water molecules.[13,14] This explains the increase in the surface tension with the addition of these salts. However, at 30% wt/vol concentration both electrolytes produced in an initial low surface tension followed by an increase with time (Figure 7). This result is related to the increased density that temporarily decreased self-diffusion as the molecules restrict each other's movements.[13-16] Consequently, at high concentrations of electrolytes, the ions attract large amount of water molecules to form "solvated cages," but during atomization they temporarily lose the cage structure and therefore cause a lower surface tension (< 200 milliseconds). Thus, electrolytes alter the solvent properties and polymer conformations that lead to the complex interplay of aerodynamic forces, and all contribute to the complexities of atomizing a multi-component nasal formulation. In conclusion, the applications of Box-Behnken experimental design facilitated the prediction and identified major excipient influences on viscosity, droplet size (Dv_{50}), and in vitro drug release.

REFERENCES

1. Dayal P, Shaik MS, Singh M. Evaluation of different parameters that affect droplet-size distribution from nasal sprays using the Malvern Spraytec. *J Pharm Sci.* 2004;93:1725–1742.

2. Malmsten M. *Surfactants and Polymers in Drug Delivery.* Lancaster, PA: Dekker; 2002.

3. Rotthäuser B, Kraus G, Schmidt PC. Optimization of an effervescent tablet formulation using a central composite design optimization of an effervescent tablet formulation containing spray dried $_L$-leucine and polyethylene glycol 6000 as lubricants using a central composite design. *Eur J Pharm Biopharm.* 1998;46:85–94.

4. Nazzal S, Nutan M, Palamakula A, Shah R, Zaghloul A, Khan MA. Optimization of a self-nanoemulsified tablet dosage form of Ubiquinone using response surface methodology: effect of formulation ingredients. *Int J Pharm.* 2002;240:103–114.

5. Poncet-Legrand C, Lafuma F, Audebert R. Rheological behavior of colloidal dispersions of hydrophobic particles stabilized in water by amphiphilic polyelectrolytes. *Colloids Surf A: Physicochemical Eng Aspects.* 1999;152:251–261.

6. Chiotelli E, Pilosio G, Le Meste M. Effect of sodium chloride on the gelatinization of starch: a multimeasurement study. *Biopolymers.* 2002;63:41–58.

7. Box GEP, Behnken DW. Some new 3 level designs for the study of quantitative variables. *Technometrics.* 1960;2:455–475.

8. Omelczuk MO, McGinity JW. The influence of thermal treatment on the physical-mechanical and dissolution properties of tablets containing poly(DL-lactic acid). *Pharm Res.* 1993;10:542–548.

9. Pillay V, Fassihi R. Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method. *J Control Release.* 1998;55:45–55.

10. Borodin O, Smith GD. Molecular dynamic simulations of poly (ethylene oxide)/LiI melts. 2. dynamic properties. *Macromolecules.* 2000;33:2273–2283.

11. van Zon A, Bel G-J, Mos B, Verkerk P, de Leeuw SW. Structural relaxation in polyethylene oxide: salt solutions. *Comp Mater Sci.* 2000;17:265–269.

12. Smitter LM, Guedez JF, Muller AJ, Saez AE. Interactions between poly(ethylene oxide) and sodium dodecyl sulfate in elongational flows. *J Colloid Interface Sci.* 2001;236:343–353.

13. Dougherty RC. Density of salt solutions: effect of ions on the apparent density of water. *J Phys Chem B.* 2001;105:4514–4519.

14. Leberman R, Soper AK. Effect of high-salt concentrations on water-structure. *Nature.* 1995;378:364–366.

15. Walrafen GE, Chu YC. Shear viscosity and self-diffusion evidence for high concentrations of hydrogen-bonded clathrate-like structures in very highly supercooled liquid water. *J Phys Chem.* 1995;99: 10635–10643.

16. Madan B, Sharp K. Changes in water structure induced by a hydrophobic solute probed by simulation of the water hydrogen bond angle and radial distribution functions. *Biophys Chem.* 1999;78:33–41.

17. Hakem F, Lal J. Polyelectrolyte-like behavior of poly(ethylene-oxide) solutions with added monovalent salt. *Europhys Lett.* 2003;64:204–210.

18. Bernson A, Lindgren J, Weiwei H, Frech R. Coordination and conformation in PEO, PEGM, and PEG systems containing lithium or lanthanum triflate. *Polym.* 1995;36:4471–4478.

19. McCallion ON, Taylor KM, Thomas M, Taylor AY. Nebulization of fluids with different viscosity and surface tension. *J Aerosol Med.* 1995;8:281–284.

20. Newman SP, Pellow PG, Clarke SW. Droplets sizes from medical atomizer (nebulizers) for drug solutions of different viscosity and surface tension. *Atomization Spray Tech.* 1987;3:1–11.

21. McCallion ON, Patel MJ. Viscosity effects on nebulization of aqueous solutions. *Int J Pharm.* 1996;130:245–249.

22. Frese Ch, Ruppert S, Sugar M, et al. Adsorption kinetics of surfactant mixtures from micellar solutions as studied by maximum bubble pressure technique. *J Colloid Interface Sci.* 2003;267:475–482.

23. Šarković D, Babović V. Experiments of water aerosol estimations of droplet parameters. *Physics, Chemistry and Technology.* 2002;2:197–208.

24. Biswas G, Som SK. Coefficient of discharge and spray cone angle of a pressure nozzle with combined axial and tangential entry of power-law fluids. *Appl Sci Res.* 1986;43:3–22.

25. Rayleigh L. On the instability of jets. *Proc Lond Math Soc.* 1878;10:4–13.

26. Squire HB. Investigation of the instability of a moving liquid film. *Brit J Appl Phys.* 1953;4:167–169.

27. Thomas GO. The aerodynamic breakup of ligaments. *Atomization and Sprays.* 2003;13:117–129.

28. Martin A. *Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences*. Philadelphia, PA: Lea & Fabiger; 1993.

29. Scatena LF, Brown MG, Richmond GL. Water at hydrophobic surfaces: weak hydrogen bonding and strong orientation effects. *Science.* 2001;292:908–912.