Rheological behavior and stability of ciprofloxacin suspension: Impact of structural vehicles and flocculating agent

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Abstract

Ciprofloxacin is a fluoroquinolone and is used against a broad spectrum of gram-negative and gram-positive bacteria. The aim of the study is to investigate the effect of structural vehicles and other formulating factors on physical stability and rheological behavior of ciprofloxacin suspension. To formulate the suspensions, the effect of glycerin and polysorbate 80 as wetting agents was evaluated. Then to achieve controlled flocculation, different concentrations of sodium chloride and calcium chloride were added. After choosing suitable wetting and flocculating agents, structural vehicles such as sodium carboxyl methyl cellulose (NaCMC), hydroxypropylmethylcellulose (HPMC) and Veegum were evaluated. Physical stability parameters such as sedimentation volume, the degree of flocculation and the ease of redispersion of the suspensions and growth of crystals were evaluated. After incorporation of structural vehicles, the rheological properties of formulations containing were also studied to find out their rheological behavior. According to the results, suspension containing glycerin (0.2% w/v) and sodium chloride (0.05% w/v) as wetting agent and flocculating agent, respectively, were the most stable formulations regarding their F and N. Microscopic observations showed the growth of crystals in ciprofloxacin suspension in formulation without excipients and the minimum amount of crystal growth was seen in suspension containing NaCMC (0.25% w/v), Veegum (0.1% w/v) and NaCl (0.05% w/v). Rheological studies showed that almost all of the formulations had psuedoplastic behavior with different degree of thixotropy. The formulation containing NaCMC (0.25% w/v), Veegum (0.1% w/v) and NaCl (0.05% w/v) was the most stable formulation. It may be concluded that by altering the amount ratios of formulation factors, the best rheological behavior and the most proper thixotropy may be achieved.

Key words: Suspension, ciprofloxacin, wetting agents, flocculating agents, rheology

INTRODUCTION

Ciprofloxacin is a second generation fluoroquinolone antibacterial and is widely used in medicine and has a broad spectrum antibacterial activity against gram-negative and gram-positive bacteria. The mechanism of ciprofloxacin action involves inhibition of bacterial deoxyribonucleic acid (DNA) gyrase, which is essential for DNA replication. Ciprofloxacin has low solubility in aqueous solution and high rate of absorption from the stomach. It has been reported that ciprofloxacin has some toxic effects on the central nervous, cardiovascular and gastrointestinal systems.[1-4]

A suspension is a dispersed system in which the internal phase consists of solid particles and the external phase is a liquid vehicle. Suspensions are best conventional dosage forms of drugs with slow dissolution rate and have patient compliance.[5] A suspension should be uniform and any sedimentation which occurs during storage should be easily redispersed on agitation. Controlled flocculating
agents and rheological modification of the suspensions are important factors in preparation of suspensions. Flocculated suspensions are settled rapidly to form large loose and easily dispersible sediments.\cite{6}

Since the nature and concentration of polymer and other excipients have a basic impact on the stability and rheological behavior of suspension, it was decided to examine the effect of different ingredients such as sodium carboxyl methyl cellulose (NaCMC), Veegum, and hydroxypropyl methyl cellulose (HPMC) on physical stability and other necessary characteristic of 5% ciprofloxacin suspension.

**MATERIALS AND METHODS**

Glycerin, polysorbate 80, sodium chloride (NaCl), calcium chloride (CaCl\(_2\)), NaCMC, and Veegum were purchased from Merck, Germany. Ciprofloxacin was obtained from Temad, Iran. HPMC was purchased from Aldrich, USA.

**Preparation of Suspensions**

Different concentrations of polysorbate 80 and glycerin were used as wetting agents. Briefly, the concentration of ciprofloxacin was 5% and wetting property of polysorbate 80 (0.1 and 0.2% w/v) and glycerin (0.1, 0.2 and 0.3% w/v) are evaluated by slug method. The samples were photographed and their contact angles were measured.

To achieve controlled flocculation different concentration of NaCl (0.01, 0.05, 0.1 and 0.2% w/v) and CaCl\(_2\) (1, 1.5, 1.75 and 2% w/v) were added to ciprofloxacin suspension and stored at room temperature. Then, the sample with high sedimentation volume and clear supernatant were selected. After choosing suitable wetting and flocculating agents, that showed the best sediment volume and improved the appearance of suspensions, different structural vehicles were added. The effect of NaCMC (0.1, 0.25 and 0.5% w/v), HPMC (0.1, 0.5% w/v), Veegum (0.5 and 1% w/v) and combination of NaCMC (0.25% w/v) and different concentrations of Veegum (0.1, 0.25 and 0.5% w/v) as structural vehicles were evaluated.

**Physical stability**

**Sedimentation volume (F)**

The ciprofloxacin suspensions were kept in a graduated cylinder after preparation. The heights of sediments were measured when there was no change after 3 days of storage. The sedimentation volume was calculated as \(F = \frac{V_f}{V_o}\) where \(F\) is sedimentation volume, and \(V_o\) and \(V_f\) are volume of sediment and volume of suspension, respectively.

**The degree of flocculation**

A 5% ciprofloxacin suspension was prepared without wetting and flocculating agents and stored at room temperature. After the volume of sediment was unchanged, the degree of flocculation was calculated as \(\beta = \frac{F}{F_{\infty}}\) where \(\beta\) is the degree of flocculation, and \(F\) and \(F_{\infty}\) are sediment volume of flocculated and deflocculated suspensions, respectively.

**The ease of redispersion**

The suspension samples were placed in glass cylinders and rotated periodically at 180 degree until thorough dispersion was achieved. The number of rotation needed for complete redispersion was recorded.

**Rheological assessment**

The rheological behavior ciprofloxacin suspensions in different formulations [Table 1] were determined using a Brookfield viscometer (LVDL-I + digital, USA with No. 2 spindle). The viscosity of samples was determined at 0.5, 1, 2, 2.5, 4, 5, 10, 20, 50 and 100 rpm after 1 min rotation at room temperature. The results were plotted as rheograms and their rheological behavior was determined by fitting on the corresponding Newtonian and non-Newtonian equations. Furthermore, the presence and extent of thixotropy were calculated by calculating the area surrounded between ascending and descending curves using the trapezoidal rule.

**Crystal growth**

The crystal growth ciprofloxacin suspensions a different formulation [Table 2] that was stored 2 months at room temperature, were examined by optical microscope (Olympus, R4, Japan) to determine the probability of, if any crystal growth (>40).

**Statistics**

Kruskal-Wallis test followed by Conover-Inman test for multiple-comparison was performed to compare of

| Formulation | NaCMC (% w/v) | NaCl (% w/v) | Veegum (% w/v) |
|-------------|---------------|--------------|---------------|
| F\(_1\)      | 0.25          | 0.05         | 0             |
| F\(_2\)      | 0.5           | 0.05         | 0             |
| F\(_3\)      | 0.25          | 0.05         | 0.1           |
| F\(_4\)      | 0.25          | 0.05         | 0.5           |
| F\(_5\)      | 0.25          | 0.05         | 0.25          |

NaCMC: Sodium carboxyl methyl cellulose, NaCl: Sodium chloride

| Formulation | NaCMC (% w/v) | NaCl (% w/v) | Veegum (% w/v) |
|-------------|---------------|--------------|---------------|
| F\(_1\)      | 0.25          | 0.05         | 0             |
| F\(_3\)      | 0.25          | 0.05         | 0.1           |
| F\(_4\)      | 0.25          | 0            | 0             |
| F\(_5\)      | 0.25          | 0            | 0             |

NaCMC: Sodium carboxyl methyl cellulose, NaCl: Sodium chloride
RESULTS AND DISCUSSION

Regarding their sedimentation volume, the degree of flocculation and contact angles the formulations containing glycerin 0.2% w/v as wetting agent were significantly better than polysorbate 80 containing formulations (P < 0.05). Although, the results indicated that effect of concentrations of wetting agents were not significant (P > 0.05). Therefore, according to median values of F, N and β for ciprofloxacin suspension in different formulations of glycerin and polysorbate 80 [Table 3], glycerin with concentration of 0.2 was selected as suitable wetting agent.

According to their F and β suspension containing 0.05% NaCl showed significant difference with other suspensions (P < 0.05). Concentration of 2% CaCl$\text{\textsubscript{2}}$ was significantly different in sedimentation volume and the degree of flocculation with concentrations of 0.01, 0.05, 0.1 and the value of F and β was less than other concentrations of CaCl$\text{\textsubscript{2}}$ and NaCl was high in compare other concentrations of CaCl$\text{\textsubscript{2}}$ (P<0.0001). According to the results of Table 4, by increasing the concentration of NaCl, the value of F and β decreases and concentration of 1% NaCl was significance different with concentrations of 0.01, 0.05 and 0.1% NaCl in its ease of redispersion (P < 0.05). Furthermore 2% CaCl$\text{\textsubscript{2}}$ was significant different with concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5% NaCl and with different concentrations of CaCl$\text{\textsubscript{2}}$ (P < 0.05). According to median values of F, N, and β NaCl (0.05% w/v) was selected as flocculating agents [Table 4].

Median values of F, N and β for ciprofloxacin suspension in different structural vehicles are shown in Table 5. Results showed that HPMC could not increase the stability of suspension and also sedimentation volume was reduced and caused coagulation. NaCMC (0.5% w/v) increased physical stability of suspension. Veegum, in comparison to NaCMC showed low stability. However, the combination of Veegum and NaCMC increased the sedimentation volume.

Results of rheological evaluation showed that all of the formulations (F$\text{\textsubscript{i}}$ - F$\text{\textsubscript{j}}$) except control suspensions and the suspension containing 0.1% HPMC and NaCl showed pseudoplastic behavior with some degree of thixotropy [Figure 1]. The value of N as indicator that defines the type of flow for different formulations is presented in Table 6. The important parameter of the flow behavior of the composite dispersion was the area of the hysteresis loop between the ascending and descending curves of the rheogram that are shown in Table 7. Evaluation of hysteresis area revealed that all of the formulations had a relatively medium thixotropy behavior that was necessary for physical stability. Control suspension and the suspension containing 0.1% HPMC and NaCl that showed anti-thixotropic behavior. It is generally accepted.

| Table 3: The median (range) of F, N and β for ciprofloxacin suspension in different formulations of glycerin and polysorbate 80 |
| Formulation % | Median (range) | F | N | β |
|----------------|----------------|---|---|---|
| Glycerin 0.1   | 0.895 (0.02)   | 4 (1) | 1.07 (0.04) |
| Glycerin 0.2   | 0.95 (0.02)    | 3 (2) | 1.13 (0.02) |
| Glycerin 0.3   | 0.90 (0.015)   | 3 (1) | 1.07 (0.02) |
| Polysorbate 80 | 0.88 (0.05)    | 2 (1) | 1.04 (0.04) |

| Table 4: The median (range) of F, N and β for ciprofloxacin suspension in different formulations of containing NaCl and CaCl$_2$ as flocculating agents |
| Formulation % | Median (range) | F | N | β |
|---------------|----------------|---|---|---|
| NaCl 0.01     | 0.90 (0.1)     | 3 (2) | 1.07 (0.05) |
| NaCl 0.05     | 0.94 (0.05)    | 3 (2) | 1.12 (0.06) |
| NaCl 0.1      | 0.90 (0.04)    | 3 (1) | 1.07 (0.05) |
| NaCl 0.2      | 0.90 (0.05)    | 4 (1) | 1.07 (0.06) |
| NaCl 0.5      | 0.88 (0.03)    | 4 (2) | 1.05 (0.04) |
| NaCl 1        | 0.88 (0.03)    | 4 (1) | 1.05 (0.03) |
| CaCl$_2$ 1    | 0.88 (0.03)    | 3 (1) | 1.04 (0.04) |
| CaCl$_2$ 1.5  | 0.88 (0.03)    | 4 (1) | 1.05 (0.04) |
| CaCl$_2$ 1.75 | 0.89 (0.02)    | 4 (1) | 1.06 (0.02) |
| CaCl$_2$ 2    | 0.86 (0.04)    | 5 (2) | 1.02 (0.05) |

NaCl: Sodium chloride, CaCl$_2$: Calcium chloride

| Table 5: Median (range) of F, N and β for ciprofloxacin suspension in different structural vehicles |
| Formulation % | Median (range) | F | N | β |
|----------------|----------------|---|---|---|
| HPMC 0.1       | 0.64 (0.08)    | 5 (1) | 0.47 (0.09) |
| HPMC 0.5       | 0.66 (0.07)    | 4 (1) | 0.79 (0.08) |
| NaCMC 0.1      | 0.95 (0.02)    | 5 (1) | 1.13 (0.02) |
| NaCMC 0.5      | 1 (0.01)       | 4 (1) | 1.19 (0.01) |
| NaCMC 0.25     | 0.98 (0.04)    | 5 (2) | 1.17 (0.05) |
| NaCMC 0.25     | 0.94 (0.01)    | 4 (2) | 1.12 (0.01) |
| Veegum 0.1     | 0.95 (0.03)    | 5 (1) | 1.14 (0.04) |
| Veegum 0.5     | 0.95 (0.03)    | 4 (2) | 1.13 (0.04) |
| Veegum 0.25    | 0.92 (0.04)    | 4 (1) | 1.01 (0.05) |
| Veegum 1       | 0.93 (0.03)    | 5 (1) | 1.01 (0.04) |

HPMC: Hydroxypropyl methyl cellulose, NaCMC: Sodium carboxyl methyl cellulose
that the greater the hysteresis area, the stronger the thixotropic property.[7]

Suspensions containing 0.5% NaCMC and NaCl showed the highest thixotropy and sedimentation volume. Also combination of Veegum and NaCMC caused a significant raise in thixotropy. By increase in concentration of Veegum, thixotropy was reduced. It is suggested that increasing of Veegum concentration may lead to the formation of molecular network and hence a rapid increase in system recovery. A good suspension should have high thixotropy and pseudoplastic behavior.[7]

Microscopic observation showed the growth of crystals in F6, the formulation in which no excipient was added [Figure 2a] and the minimum amount of crystal growth was seen in F3, after 2 months of storage [Figure 2b].

According to the results, HPMC could not improve thixotropy behavior of suspensions, while property thixotropy behavior observed in the formulations containing NaCMC. Also when NaCl as flocculating agent in formulations containing NaCMC was added, the

Table 6: Amount of N for evaluation of type of behavior for different formulations

| No. formulation | N  |
|-----------------|----|
| F1              | 1.77|
| F2              | 1.64|
| F3              | 1.78|
| F4              | 2.57|
| F5              | 1.77|

Table 7: Median (range) of the hysteresis loop of different formulations

| Formulation | Median (range) of the hysteresis loop |
|-------------|--------------------------------------|
| F1          | 920.9 (124.3)                        |
| F2          | 1522 (354.0)                         |
| F3          | 919.4 (311.9)                        |
| F4          | 314.6 (530.4)                        |
| F5          | 388.6 (259.0)                        |
| F6          | −2229 (772.8)                        |
| F8*         | −2455 (418.0)                        |

*F8: HPMC 0.1%, NaCl 0.05%

Figure 1: Rheograms and thixotropy of ciprofloxacin suspensions in a vehicle containing, (a) F1, (b) F2, (c) F3, (d) F4, (e) F5 are showing their rheological behaviors and thixotropies

Figure 2: Microscopic view of crystal growth ciprofloxacin suspension without excipients (left) and sodium carboxyl methyl cellulose 0.25%, veegum 0.1% and sodium chloride 0.05% (right) (×40)
Rheological behavior of suspensions and sedimentation volume were improved. In addition, presence of Veegum in formulations containing NaCMC and NaCl was necessary, because the minimum amount of crystal growth was observed. It is suggested that Veegum is adsorbed at the particle surface and retarded crystal growth.

Rheological properties of suspensions depend on their particle size, shape and surface modification.[6] Moreira et al. in 2010 investigated the influence of oleic acid on the rheology and in vitro release of lumiracoxib from poloxamer gels. The results of rheological study showed a pseudoplastic behavior, and the increase of poloxamer from 20% to 30% w/w and oleic acid increased the viscosity of the gel.[6] Li et al. in 2005 reported hydroxyethyl cellulose in combination with bromododecane increased viscosity and caused thermal stability.[7] Gallardo et al. in 2006 investigated rheological properties of ethylcellulose latex. The results showed that viscoelastic behavior was affected by temperature. High temperature also caused a change in particle shape.[7] Viriyaroj et al. in 2009 reported that carboxymethyl cellulose, methylcellulose and xanthan gum used as a suspending agent caused desirable rheological behavior Xanthan gum exhibits plastic or pseudoplastic flow and this behavior was possibly a result of shearing action on the long chain molecule of xanthan gum.[8] Khunawattanakul et al. in 2008 investigated the rheology, flocculate size and zeta potential of the chitosan, a positively charged polymer, and magnesium aluminum silicate, negatively charged clay dispersions. The results showed the electrostatic interaction between chitosan and magnesium aluminum silicate caused a change in flow behavior and flocculation of the composite dispersions, depending on the high and low molecular weights of chitosan (high chitosan and low chitosan, respectively). Increasing hysteresis areas of the composite dispersion were found when MAS was added, that increased in thixotropic properties. Heat treatment also caused a decrease of viscosity and hysteresis area of the high chitosan – magnesium aluminum silicate dispersion. The decrease in viscosity of the composite dispersion might be caused by a reduction of intra-molecular hydrogen bonding of chitosan and a decrease in hydrogen-bonded hydration of chitosan, while heating did not affect the hydration of magnesium aluminum silicate, because magnesium aluminum silicate dispersions were prepared and hydrated using hot water in the preparation process.[8]

CONCLUSION

The combination of glycerin (0.2%) and NaCl (0.05%) were selected as the best wetting and flocculating agents. Rheological studies showed that all of the formulations were pseudo-plastic with different degrees of thixotropy. Microscopic observation showed the growth of crystals in control ciprofloxacin suspension. The minimum amount of crystal growth was seen in suspension containing NaCMC (0.25% w/v), Veegum (0.1% w/v) and NaCl (0.05% w/v). In conclusion, in different formulations of ciprofloxacin suspension regarding their F, N and β, the suspension containing NaCMC (0.25% w/v), Veegum (0.1% w/v) and NaCl (0.05% w/v) (Formulation F,3) was the most physically stable formulation.

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