Isoxsuprine Hydrochloride Loaded Cellulose Acetate Phthalate Microsponge Drug Delivery System: Design and Evaluation

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ABSTRACT

Aim: The present study was aimed to Design and evaluation of microsponge based drug delivery system of Isoxsuprine Hydrochloride. The microsponge drug delivery system is designed for site specific and controlled release of drug by using cellulose acetate phthalate to improve the site-specific absorption of drug. Materials and Methods: The microsponges was formulated by modified quasi emulsion solvent diffusion technique. The chemical interaction between Isoxsuprine Hydrochloride, cellulose acetate phthalate, ethyl cellulose and polyvinyl pyrrolidone was studied by FTIR, the results of FTIR it was confirmed that there were no chemical reaction in between drug and polymer. The compatibility study of drug and polymer were confirmed by DSC. Results: The in vitro drug release found in between range of 91.97% to 98.78% the highest % CDR was shown by formulation MS5. The optimized formulation (MS5) demonstrated favorable % entrapment efficiency (93.6%), % buoyancy (78%) and % cumulative drug release (98.78%). SEM revealed the release of Isoxsuprine Hydrochloride in controlled release pattern from spherical and porous microsponges. Conclusion: This study provides a new approach to formulate and evaluate the microsponges of Isoxsuprine Hydrochloride for treatment of premature labor during pregnancy. Key words: Microsponges, Isoxsuprine Hydrochloride, Cellulose acetate phthalate, Quasi emulsion solvent diffusion, Site specific absorption.

INTRODUCTION

Administration of the drug through the oral route is most convenient, economical and common. Although the oral drug delivery system suffers from problem with absorption, short half-life as well as elimination. These problems can be avoided by formulating orally controlled release formulation for slow release of the drug into the gastrointestinal tract. This route can be easily utilized for the development of sustained and controlled release formulation of drugs. The oral route of drug delivery is most selective because of its economy, convenience and huge patient acceptance. The commonly used solid dosage forms are capsule and tablet; formulated nanoparticles, microparticles, microsphere, microsponge and nanospheres may be administered to a patient in the form of tablets and capsule. Microsponge drug delivery system is patented highly cross-linked, porous, a polymeric system that can entrap a broad range of active ingredients and release them by enhancing performance. Microsponges are mostly used for topical controlled release drug delivery system, but recently it can be used for oral drug delivery with solid dosage forms like tablet and capsule. Microsponge drug delivery system offers delivery of drug with a minimum dose, enhanced stability, least side effects and controlled release of the drug. Polymers
used in microsponge can entrap for benefits such as enhanced product efficacy, tolerability and mildness. Although the size of microsponge may vary from 5-300 μm, typical 25μm sphere can have up to 250000 pores. Now a day microsponge delivery System is used in cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products. There are several advantages of microsponge like stability at the pH range 1-11, stable at the temperature up to 130°C, compatible with most of the vehicle and active ingredients, the average size of microsponge pore is only 0.25μm and cost-effective.5,7

IUPAC nomenclature of Isoxsuprine Hydrochloride is 1RS, 2SR)-1-(4-hydroxyphenyl)-2-[(1RS)-1-methyl-2-phenoxyethylamino] propan-1-ol hydrochloride.8 Isoxsuprine hydrochloride is an orally effective long-acting β - receptor stimulant which has direct smooth muscle relaxant property. It has been used as uterine relaxant for threatened abortion and dysmenorrhea.9 Several attempts were made to formulate microsponge drug delivery systems by different method of preparation like emulsion solvent diffusion method10 and quasi-emulsion solvent diffusion method11 for Fluconazole3 Ketoprofen12 Benzoyl peroxide10 Flurbiprofen11 Meloxicam13 Metronidazole14 Lansoprazole,13,16 Flurbiprofen,17 Curcumin,18 Dicyclomine19 Oxybenzone.20

In the present work Quasi-emulsion technique was implimented for formulation of microsponge drug delivery system of isoxsuprine hydrochloride. Optimized formulation was characterized using particle size analysis, differential scanning calorimetric analysis (DSC), Fourier transform infrared analysis (FTIR) and Scanning Electron Microscopy (SEM). The formulated batches were evaluated for product yield, particle size, drug content, entrapment efficiency, in vitro buoyancy and in vitro drug release. The obtained in vitro drug release data was fitted to empirical equations to understand the mechanism and kinetics of drug release.

**MATERIALS AND METHODS**

**Materials**

Isoxsuprine Hydrochloride I.P., was obtained as a gift sample from Jayco chemicals Industries, Thane. Cellulose acetate phthalate, ethyl cellulose, polyvinyl pyrrolidone, ethanol, chloroform, span 80, hydrochloric acid and potassium dihydrogen phosphate were procured from loba chemicals, Mumbai. A measured quantity of Isoxsuprine hydrochloride, Ethyl cellulose and cellulose acetate phthale were added in the ethanol and chloroform (organic phase). For the preparation of 1% (w/v) dispersion span 80 was added in above solution with continuous agitation. The solution was uniformly emulsified in polymeric solution to prepared w/o emulsion. Polyvinyl pyrrolidone was used for preparation of aqueous phase in a separate beaker, prepared emulsion (w/o) was emulsified in it. A magnetic stirrer was used for continuous string of this w/o/w emulsion for 8 hr. The dispersed droplets were solidified in the aqueous phase by solvent evaporation. The microsponge was filtered, dried at 60°C in the hot air oven and stored in a desiccator. For more detail refer to Table 1.

**Evaluation**

**Preformulation studies of isoxsuprine hydrochloride**

**Identification**

0.01% w/v solution of isoxsuprine hydrochloride in 0.1M Hydrochloric acid was analysed at the range of 230 nm to 360 nm. Two maxima observed at about 269 nm and 274 nm with absorbance 0.73 and 0.72 respectively.

**Solubility**

An excess amount of isoxsuprine hydrochloride was dissolved in water in a volumetric flask with frequent shaking and sonicating for 1hr. The saturated solution was filtered through whatman filter paper and the filtrate was suitably diluted with distilled water and analyzed by spectrophotometrically (Systronics, Ahmedabad.) at 269 nm against a blank. The study was performed in triplcate.

**Melting point**

For the determination of melting point small amount of drug was taken in capillary tube closed at one end It was placed in melting point apparatus (lab intelligence appliances) and the melting point was corrected.

**Infrared spectroscopy (IR) study**

Bruker Alpha –T spectrophotometer was used to determine IR spectrophotometric study of drug and polymer. The selenium bromide method was used to study IR spectra of drug and polymer to rule out drug - excipients interaction n.

**Evaluation of Microsponges**

**Particle size and Product yield**

The optical microscope was used to determine the particle size. The mean particle size of more than
300 particles were calibrated against calibrated ocular micrometer. Product yield of microsponges was calculated by dividing the weight of microsponges to the total amount of material used in the preparation of microsponges.

**Drug content and entrapment efficiency**

Isoxsuprine hydrochloride loaded microsponges of each batch equivalent to 10 mg of Isoxsuprine hydrochloride were weighed and powdered using glass mortar and 10 mg powder was taken in to a volumetric flask and extracted with 5 mL of acetone by vortexing. The sample was centrifuged at 2000 rpm for 10 min, then the solution was filtered and assayed spectrophotometrically at 269 nm by using double beam UV-Spectrophotometer (Systronics, Ahmedabad). Drug content was calculated by dividing the practical drug content to the theoretical drug content. Entrapment efficiency was determined by dividing the practically entrapped amount of drug to the total amount of drug.

**In vitro buoyancy**

In this test, the accurately measured quantity of microsponge 50 mg was spread on the surface of the beaker containing 100 ml phosphate buffer (pH 4.5) and 0.02% Tween 80 for wetting effect. The magnetic stirrer was used for continuous stirring of medium for 8hr. At the end number of floating microsponges were measured.

**In-vitro drug release study**

United states Pharmacopoeia (USP) dissolution testing apparatus 2 (Paddle type Electrolab USP TDL-08L, India). was used for the in vitro extended release of isoxsuprine hydrochloride from microsponge by using treated dialysis membrane. In this test 900 mL of simulated phosphate buffer having pH 4.5 was used as dissolution medium. The content was rotated at 50 rpm at 37 ± 1°C. A 5 mL sample of the solution was withdrawn from the dissolution medium initially and then regularly after 30 min interval up to 8 hr and analyzed drug release spectrophotometrically at 269 nm. All the readings were performed in triplicate.

**Characterization of optimized formulation**

**Differential scanning calorimetry (DSC)**

Differential scanning calorimetry (SDT Q600 V20.9 Build 20) was used for estimation of thermal behavior of isoxsuprine hydrochloride and optimized formulation (MS5) to identify the physical compatibility of drug with the polymer matrix. The samples were sealed in aluminum pans heated in an atmosphere of nitrogen and thermograms were obtained by heating at a constant heating rate of 10°C/min in the range of 0–300°C. A nitrogen purge (20 ml/min) was maintained throughout the run. The instrument was equipped with an intercooler to assess the thermal behavior of the sample. Alumina standard was used to calibrate the temperature and enthalpy scale of the instrument.

**Scanning electron microscopy**

Scanning electron microscope (Mira 3 TESCAN) was used to visualize the surface morphology of pure drug and optimized formulation MS5. Photographs at definite time intervals from dissolution study were taken to predict the possible release pattern of drug delivery system before and after drug release.

**RESULTS AND DISCUSSION**

**Preformulation study of isoxsuprine hydrochloride**

In the preformulation study, the physical and chemical properties of drug and polymer were evaluated for the development of a drug carrier system in the view of its stability and bioavailability. Preformulation parameters are useful for detection of safety, efficacy, acceptability and stability of the product. The essential physicochemical properties of a drug like a solubility and melting point were determined and found to be in an acceptable range.

**Identification**

UV spectrophotometer (Systronics, Ahmedabad, India) was used for the identification of isoxsuprine hydrochloride at λmax 268.2 nm and 273.8 nm of which absorbance were found to be 0.71 and 0.69 respectively.

**Solubility**

The solubility of isoxsuprine hydrochloride in distilled water found to be 1.9%w/v.

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**Table 1: Formulation of isoxsuprine hydrochloride microsponges by Quasi-emulsion method.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Isoxsuprine hydrochloride (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>Cellulose acetate phthalate (mg)</th>
<th>Polyvinyl pyrrolidone (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-1</td>
<td>80</td>
<td>100</td>
<td>200</td>
<td>0.5</td>
</tr>
<tr>
<td>MS-2</td>
<td>80</td>
<td>100</td>
<td>400</td>
<td>0.5</td>
</tr>
<tr>
<td>MS-3</td>
<td>80</td>
<td>100</td>
<td>600</td>
<td>0.5</td>
</tr>
<tr>
<td>MS-4</td>
<td>80</td>
<td>100</td>
<td>200</td>
<td>1</td>
</tr>
<tr>
<td>MS-5</td>
<td>80</td>
<td>100</td>
<td>400</td>
<td>1</td>
</tr>
<tr>
<td>MS-6</td>
<td>80</td>
<td>100</td>
<td>600</td>
<td>1</td>
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</table>
Melting point
The melting point of the drug was determined using melting point apparatus and the melting point was found 202°C.

Infrared spectroscopy (IR) study
The prominent peaks of isoxsuprine hydrochloride were observed in the region of 3362 cm⁻¹ due to the -OH stretching, a peak at 3292 cm⁻¹ due to N-H stretching and a peak 3072 cm⁻¹ due to aromatic C-H stretching. Peaks at 2981 cm⁻¹ showed the presence of aliphatic C-H stretching, 1577 cm⁻¹ and 1454 cm⁻¹ due to C=C aromatic stretching (Figure 1). The IR of a physical mixture of the drug with Ethyl cellulose and cellulose acetate phthalate showed the shape and location of peaks in the region 3362 cm⁻¹ – OH stretching, a peak at 3292 cm⁻¹ due to N-H stretching had noticeably changed. These suggest that drug interactions were absent (Figure 2).

Evaluation of Microsponges

Product yield
The product yield of microsponges was found in the range of 71.4% to 89.1% as shown in Table 2. From the experimental result, it is cleared that the product yield of microsponge is depended on the concentration of cellulose acetate phthalate and polyvinyl pyrrolidone. The highest yield of microsponge was found in formulation MS 6 (89.1%) where cellulose acetate phthalate (600 mg) and polyvinyl pyrrolidone (1 mg) were in the highest concentration. In contrast to this result lowest yield of microsponge was found in formulation MS1 (71.4%) at a low concentration of cellulose acetate phthalate (200 mg) and polyvinyl pyrrolidone (0.5mg) and which proved that the yield of microsponge is based on the concentration of cellulose acetate phthalate and polyvinyl pyrrolidone. In addition to this, at a constant concentration of polyvinyl pyrrolidone, on increasing the concentration of cellulose acetate phthalate the product yield increased as explained by Lee et al. High level of cellulose acetate delays polymer precipitation by retarding the diffusion of organic phase to aqueous phase and provides more time for droplet formation whereas at another experimental condition low level of cellulose acetate phthalate forms less viscous organic phase which causes rapid mixing and faster removal of solvent and hence decreases the yield.

The increase in product yield was observed in formulation MS4, MS5 and MS6 due to the formation of large droplets at the high level of polyvinyl pyrrolidone.

Particle size
The formulated microsponge of isoxsuprine hydrochloride having a size range in between 90.2 µm to 178.5 µm (Table 2). Formulation MS 6 exhibited a maximum particle size of 178.5 µm. The particle size of isoxsuprine hydrochloride loaded microsponge was found to be dependent on the concentration of the polymer. The quantity of cellulose acetate phthalate governs the size of microsponge, an increase in the concentration of cellulose acetate phthalate increases the particle size. As the concentration of polyvinyl pyrrolidone increases, the formation of larger microsponge takes place due to the formation of large emulsion droplets which was observed in the formulations MS4, MS5 and MS6 where particle size was 106.5 µm, 130.5 µm 178.5µm respectively.

Drug content
Drug content for all the formulation was in the range of 79.1% (MS1) to 92.3% (MS5). MS5 formulation shows the highest drug content. For more detail refer Table 2.

Entrapment efficiency
The maximum entrapment efficiency was showed by formulation MS5 (93.6%) and the minimum entrapment efficiency was showed by formulation MS1 (80.7%). The entrapment efficiency increased up to a certain limit by increasing the concentration of cellulose acetate phthalate, but beyond the certain limit of cellulose acetate phthalate it decreased, at a constant concentration of poly vinyl pyrrolidone. The best entrapment efficiency was observed in formulation MS2 (91.5%) and MS5 (93.6%) (Table 2) where the
concentration of cellulose acetate phthalate and PVP were at an intermediate level. The high value of entrapment efficiency associated with the porosity of microsponges.

**In vitro buoyancy**

In vitro buoyancy studies revealed that the microsponges formed with a high level of cellulose acetate phthalate was having less buoyancy. The formulation MS6 showed the lowest buoyancy 72% with the highest concentration of cellulose acetate phthalate and polyvinyl pyrrolidone where as the formulation MS1 showed highest 86 % with the lowest concentration of cellulose acetate phthalate and polyvinyl pyrrolidone. (Table 2) As explained by V.S. Mastiholimath et al. at higher polymer concentration the relative density of microparticles was higher and it has less porosity. In our studies, less buoyancy were found in microsponges having higher polymer concentration and higher buoyancy was found in microsponge with low polymer concentration. The results revealed microsponge with higher buoyancy found to be more porous than the microsponge with lower buoyancy.

**Table 2: The Physicochemical Evaluation of Isoxsuprine Hydrochloride Microsponge.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Product yield (%)</th>
<th>Particle size (µm)</th>
<th>Drug content (%)</th>
<th>Entrapment efficiency (%)</th>
<th>Buoyancy (%)</th>
<th>Cumulative % Drug release</th>
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</thead>
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<tr>
<td>MS1</td>
<td>71.4</td>
<td>90.2</td>
<td>79.1</td>
<td>80.7</td>
<td>86</td>
<td>95.59</td>
</tr>
<tr>
<td>MS2</td>
<td>75.3</td>
<td>95.4</td>
<td>88.9</td>
<td>91.5</td>
<td>82</td>
<td>95.61</td>
</tr>
<tr>
<td>MS3</td>
<td>82.9</td>
<td>102.4</td>
<td>82.7</td>
<td>85.1</td>
<td>74</td>
<td>91.94</td>
</tr>
<tr>
<td>MS4</td>
<td>73.2</td>
<td>106.5</td>
<td>80.6</td>
<td>82.9</td>
<td>80</td>
<td>94.32</td>
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<tr>
<td>MS5</td>
<td>78.7</td>
<td>130.5</td>
<td>92.3</td>
<td>93.6</td>
<td>78</td>
<td>98.78</td>
</tr>
<tr>
<td>MS6</td>
<td>89.1</td>
<td>178.5</td>
<td>87.2</td>
<td>86.2</td>
<td>72</td>
<td>93.09</td>
</tr>
</tbody>
</table>

In vitro drug release

The cumulative percentage drug release (% CDR) of isoxsuprine hydrochloride after 8 hr. was found between the ranges of 91.97% to 98.78% the highest % CDR was shown by formulation MS5. (Table 3) The in vitro drug release data revealed the polymer dependent drug release, the formulation with the low concentration of cellulose acetate phthalate showed the better release of drug as compared to the formulation with high concentration of cellulose acetate phthalate. The release data showed the ‘burst release’ of formulation MS1 at 2 hr. it showed the percentage release of drug 47.06% this bulk release of drug occurred due to the low concentration of cellulose acetate phthalate and presence of drug near to surface of microsponge. Formulation MS5 showed better % CDR, as it was having intermediate concentration of cellulose acetate phthalate and polyvinyl pyrrolidone which allowed release of drug in controlled pattern, it can also be correlated with the particle size of microsponge, as explained by Geetika Wadhwa et al. The microsponge with large particle size are having less % CDR and microsponge having small size show the greater % CDR because it has the greater surface area than large microsponge. The data obtained from in vitro drug release was plotted on a graph (Figure 3a and 3b) by taking time on X-axis and % CDR on the Y-axis.

**Table 3: Percentage cumulative drug release (% CDR) of Isoxsuprine Hydrochloride loaded microsponges.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Time in h.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>MS1</td>
<td>24.30</td>
<td>47.06</td>
<td>61.99</td>
<td>69.71</td>
<td>77.46</td>
<td>84.24</td>
<td>89.93</td>
<td>95.59</td>
<td></td>
</tr>
<tr>
<td>MS2</td>
<td>20.79</td>
<td>28.51</td>
<td>40.26</td>
<td>50.14</td>
<td>59.14</td>
<td>67.57</td>
<td>83.09</td>
<td>95.61</td>
<td></td>
</tr>
<tr>
<td>MS3</td>
<td>15.11</td>
<td>34.71</td>
<td>46.60</td>
<td>55.77</td>
<td>65.67</td>
<td>72.20</td>
<td>77.33</td>
<td>91.94</td>
<td></td>
</tr>
<tr>
<td>MS4</td>
<td>22.68</td>
<td>39.77</td>
<td>51.28</td>
<td>61.90</td>
<td>71.18</td>
<td>81.18</td>
<td>88.76</td>
<td>94.32</td>
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<tr>
<td>MS5</td>
<td>16.42</td>
<td>26.24</td>
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<td>76.20</td>
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<tr>
<td>MS6</td>
<td>21.98</td>
<td>36.35</td>
<td>42.28</td>
<td>46.78</td>
<td>55.53</td>
<td>62.23</td>
<td>78.29</td>
<td>93.09</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3: In vitro drug release of microsponge formulations 3a. MS1, MS2 and MS3, 3b. MS4, MS5, MS6.
Kinetic modeling of microsponge formulation

The in vitro drug release data of isoxsuprine hydrochloride loaded microsponge was subjected to various release models like Higuchi, Korsmeyer-Peppas, zero order and first order and the best fit model was decided by highest $R^2$ values. The highest $R^2$ values were shown by Higuchi, so the best model for optimized formulation was Higuchi. The highest $R^2$ values were observed in the Higuchi model (0.9970 for MS5), by the highest $R^2$ values 0.931 Fickian diffusional releases occurs by the general molecular diffusion of the drug due to a chemical potential gradient. The Korsmayer-Peppas plot of MS5 formulation showed a Fickian release pattern with correlation coefficient of $R^2 = 0.931$. The Figure 4 a, b, c and d. Showed graph of the optimized formulation after comparing the various kinetic models.

DSC Analysis

Initial slight endothermic bend was observed in DSC of isoxsuprine hydrochloride (Figure 5) at 85.33°C afterward a sharp endothermic peak begins at 213.84°C which was probably due to melting of Isoxsuprine Hydrochloride (M.P-203°C). This peak was observed until 223.79°C. Then system stopped absorbing heat this might be due to melting of total drug The (Figure 6) explains about DSC thermogram of MS 5 formulation although it has shown endothermic peak at lower temperature 82.95°C the peak is broad and not sharp and the calorie consumption at this temperature is much higher than that of isoxsuprine hydrochloride thermogram. Afterward, the DSC of formulation shows a slight peak at 163.51°C and 177.49°C and then the peak is shifted towards high temperature 199.28°C to 215.63°C even no significant change in temperature of the drug was observed with the MS5 formulation so, isoxsuprine hydrochloride is found to be compatible with the polymers used in this formulation.

SEM Analysis

SEM Analysis was carried out to determine surface morphology of the formulations before and after the drug dissolution from the optimized (MS5) formulation. This study revealed that change in the surface morphology of MS5 formulation from 4 hr to 8 hr. in dissolution medium shown in Figure 7 (a, b and c ). The erosion of polymer of microsponge is clearly observed in (Figure 7 d) after 8 hr. Hence, we can say that there is swelling as well as erosion mechanism involved in this drug delivery system.

CONCLUSION

Microsponge as a drug delivery system is evolving technology having broad application in the field of pharmaceutical manufacturing. Microsponges are porous microparticles having the ability to release the drug in a controlled manner; it can be productively used in targeting the drug to their desired sites of action.
Thus, microsponges are utilized as a drug delivery system for targeted and controlled release of different oral, topical and site-specific drug delivery system.

The microsponge particles have the ability to adhere to the surface of intestinal mucosa so, it is applied for local drug delivery. Preparation methods of microsponge are adjustable according to drug-polymer ratio; which is proved to be as safe and stable drug delivery system for oral and topical drug delivery system.

The model drug isoxsuprine hydrochloride used in the present study having the vasodilator activity. It is also used as smooth muscle relaxant of uterine. The positive results from this study can be utilized for formulation novel drug delivery of isoxsuprine hydrochloride at industrial scale.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors will declare no conflict of interest.

ABBREVIATIONS

OTC: Over the counter; FTIR: Fourier-transform infrared Spectroscopy; DSC: Differential scanning calorimetry; % CDR: Percentage cumulative drug release; SEM: Scanning electron microscopy; mg: Milligram; h: Hours.

REFERENCES

2. Won R, inventor; Heron Therapeutics LLC, assignee. Method for delivering an active ingredient by controlled time release utilizing a novel delivery vehicle which can be prepared by a process utilizing the active ingredient as a porogen. United States patent US 4,690,825. 1987.
In the present study microspoon drug delivery system of Isoxsuprine Hydrochloride, a vasodilator used for smooth muscle relaxation to avoid the premature labor pain and dysmenorrhea. This formulation was prepared by using cellulose acetate phthalate, Polyvinyl pyrroldione and ethyl cellulose. Quasi-emulsion technique was implemented for the preparation of Isoxsuprine Hydrochloride microspoon.

The prepared batches of formulations were evaluated for FTIR, DSC, SEM and in vitro dissolution studies. The results of this work reveals the applicability of microspoon as a drug delivery system for site specific absorption of drug by applying the listed polymers.
About Authors

Shubhangi Kagade was a Post Graduate Student of Solapur University Solapur. My area of work is Novel Drug Delivery System. Currently I am working at Ciantha Research Center Ahmedabad in Pharmacovigilance Department.

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