Original Article

Formulation and Evaluation of Indomethacin Solid Dispersion by using Hydrophilic Polymers

Rabi Narayan Panigrahy*, Krishna Mohan Chinnala

School of Pharmacy, Nalla Narasimha Reddy Group of Institutions, Ghatkesar, RR Dist, Hyderabad, Andhra Pradesh, India - 500088

ARTICLE INFO

Objective: The objective of the study was to formulate & evaluate Solid Dispersions of a poorly water soluble drug like Indomethacin, as model drug by using hydrophilic polymers like Polyethylene glycol-4000 and Polyvinyl Pyrrolidine K30.

Experimental approach: Melting fusion Method was employed for the preparation of Solid Dispersion of Indomethacin. The various batches of prepared Solid Dispersion using PEG-4000 and PVP K30 were subjected for drug content analysis. The prepared Solid Dispersions were characterized by Fourier transformed infrared spectroscopic analysis (FT-IR), to ascertain for any interaction between the drug and the polymers used and to confirm the encapsulation of the drug with in polymer matrix.

Findings & Discussions: All Solid Dispersion Formulations satisfies the dissolution acceptance level ($\text{Q}_{\text{min}}$=75%), where B3 shows better resistance to drug degradation in dissolution medium pH 7.4 phosphate buffer. Percentage cumulative drug release was 87.11 % within 40 minute in the PEG (1:5) batch (for pure drug only 77 %), which was maximum release rate achieved among all batches, so exposure time of Indomethacin to the dissolution medium is very less. Tablet shows good release, up to 86.98 % of the drug was released to the dissolution media within 40 minute. For the immediate release tablet dissolution acceptance level ($\text{Q}_{\text{min}}$=75%) which was satisfied.

Conclusion: The tablets of that batch PEG (1:5) had considerable content uniformity with good drug release pattern. Thus From the discussion it was concluded that PEG increases the solubility of Indomethacin as compared to PVP K30 at 1:5 drugs to polymer ratio.

Corresponding author *
E-mail address: rabi.papu@gmail.com (Rabi Narayan Panigrahy)
INTRODUCTION
The poorly water-soluble drug candidates often emerge from contemporary drug discovery programs, and present formulators with considerable technical challenges. The poor solubility and low dissolution rate of poorly water-soluble drugs in the aqueous gastro-intestinal fluids often cause insufficient bioavailability. Especially for class II substances according to the Bio-pharmaceutics Classification System (BCS), the bioavailability may be enhanced by increasing the solubility and dissolution rate of the drug in the gastro-intestinal fluids. The most feasible approaches for achieving major solubility and dissolution are micronization, complexation, solid dispersion, salt form of drug, using of surfactants etc. Enhancement of bioavailability of hydrophobic drugs is one of the major challenges in drug development. Of the plethora of pharmaceutical technologies available to address this issue, Solid Dispersion is one of the useful methods for the dispersion of the drug into an inert, hydrophilic polymer matrix. Solid Dispersions in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and hence possibly bioavailability of a range of hydrophobic drugs. Although a large number of studies have been published but the mechanisms underpinning the observed enhancement of the rate of drug release are not yet understood. The use of Solid Dispersions as an effective source of improving the dissolution rate of poorly soluble drugs has been well studied and demonstrated. The poorly water-soluble drugs are characterized by insufficient bioavailability (low dissolution rates) and absorption in the gastro-intestinal tract. Different methods have been used to increase the dissolution and bioavailability of poorly water-soluble drugs including micronization, the use of surfactants, and the formation of Solid Dispersions. Solid Dispersions display an enhanced solubility of drug because of the conversion of the drug’s crystal lattice into an amorphous form, particle size reduction and increased wettability by the hydrophilic polymer. Solid Dispersion (SD) technique has been widely used to improve the dissolution rate, solubility and oral absorption of poorly water-soluble drugs. Solid Dispersion is defined as the dispersion of one or more active ingredients in an inert excipients or matrix (carrier), where the active ingredients could exist in finely crystalline, solubilised or amorphous state. Once the Solid Dispersion is exposed to aqueous media and the carrier dissolve, the drug is released as very fine to colloidal particles. Because of greatly enhanced surface area obtained in this way, the dissolution rate and the bioavailability of poorly water-soluble drugs are expected to be high. Various pharmaceutical approaches for the preparation of SDs include co-precipitation, lyophilization, spray drying, melting solvent method, melt extrusion method, solvent evaporation, fusion and powder mixing methods. Melting and solvent evaporation methods are the two major processes of preparing Solid Dispersions. Solid Dispersions appear to be a better approach to improve drug solubility than other techniques, because they are easier to produce and more applicable. For instance, salt formation can only be used for weakly acidic or basic drugs and not for neutral. Furthermore, it is common that salt formation does not achieve better bioavailability because of its in vivo conversion into acidic or basic forms. The methods that have been used to characterize Solid Dispersions are Dissolution testing, Thermo-analytical methods, differential thermo-analysis and hot Stage microscopy, Calorimetric analysis of the solution or melting enthalpy for calculation of entropy change, X-ray diffraction, Spectroscopic methods, e.g. IR spectroscopy, Microscopic methods including polarization microscopy and Scanning electron
microscopy. Knowledge with solid dispersions over the last few decades indicates that this is a very lucrative approach to improving the release rate and oral bioavailability of hydrophobic drugs. Two trends powerfully favor an increasing role for solid dispersions in pharmaceutical development: the growing number of drug candidates which are poorly soluble, and the considerable improvements in the manufacturing techniques for solid dispersions that have been made in the last few years. Another advantage of solid dispersions over other approaches is that many of the carriers that can be applied are already extensively used in the pharmaceutical industry as excipients, no toxicity studies are required. This thesis provides information to the use of solid dispersions for the development of the release rate and oral bioavailability of poorly water soluble drugs, by careful choice of the carrier it is also feasible to delay or slow down the release pattern of a drug by formulating it into solid dispersion. Principal therapeutic effect of Indomethacin is derived from their ability to inhibit prostaglandin production by inhibiting enzymatic activities in the prostaglandin synthesis. The 1st enzyme is prostaglandin synthetase or fatty acid cyclooxygenase. This enzyme converts arachidonic acid to the unstable intermediates PGG2 and PGH-2. It is now appreciated that there are two forms of cyclooxygenase, termed cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)12.

2. MATERIALS AND METHODS

2.1 Material

The Indomethacin was obtained as gift sample from Super pharma products, Ujjain. Polyethylene glycol-4000 and Polyvinyl pyrrolidine K30 were gift sample from Ozone international, Mumbai. Starch was obtained as gift sample from Merck Limited, Mumbai. Ethanol was obtained from Loba Chemie, Mumbai. All other reagents and chemicals used were of analytical reagent grade.

2.2 Preparation of Solid Dispersion

Melting fusion Method was employed for the preparation of Solid Dispersion of Indomethacin using PEG-4000 and PVP K-30. The proportion of various components used in the formulation is given in table 1.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug: Polymer ratio</th>
<th>Indomethacin</th>
<th>PEG-4000</th>
<th>PVP K30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>01:01</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>01:03</td>
<td>100</td>
<td>300</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>01:05</td>
<td>100</td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>01:01</td>
<td>100</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>01:03</td>
<td>100</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>01:05</td>
<td>100</td>
<td>-</td>
<td>500</td>
</tr>
</tbody>
</table>

Procedure in brief, the drug and the polymer were heated until the polymer melts. The molten mixture was stirred until the drug was dissolved completely in the melt and a homogeneous solution was obtained. The solution was brought to solidification by pouring it into tablet moulds under ambient conditions preferably at very low temperatures for rapid solidification. Different formulations were prepared by varying the concentration of polymer. The drug concentration was kept constant i.e. 100mg while the concentration of polymer was varied from 100mg to 300mg to 500mg giving the formulation with drug : polymer ratio 1:1, 1:3 and 1:5 respectively. The prepared Solid Dispersions were further subjected to characterization and evaluation respectively.

2.3 Evaluation of Solid Dispersion

2.3.1 Determination of Drug Content of Solid Dispersion

The various batches of prepared Solid Dispersion using PEG-4000 and PVP K30 were subjected for drug content analysis. Accurately weighed Solid Dispersion sample containing 100mg of drug were mechanically powdered and passed through sieve no (85/120). The powder retained on the sieve 120 was dissolved in
adequate quantity of phosphate buffer solutions (pH 7.4). The solution was filtered using filter paper. From this 10 ml was pipette out into a 100 ml standard volumetric flask and made up to the volume with pH 7.4 phosphate buffer solutions and estimated spectrophotometrically using UV-spectrophotometer for drug content at 320 nm. The drug content was estimated for 4 samples from the same batch for each ratio and for each method. The drug loading of the prepared microspheres was evaluated using the formula:

\[
\text{Drug loading} = \left( \frac{\text{weight of the drug in Solid Dispersion}}{\text{Solid Dispersion sample weight}} \right) \times 100
\]

2.3.2 Micromeritic Evaluation Solid Dispersion

Bulk density & Tapped density

5gm of Solid Dispersion weighed accurately & poured in a 50 ml measuring cylinder, the fluff volume was noted. Then tapped for 100 times from a uniform height and the tapped volume were determined.

\[
\text{Density} = \frac{\text{Mass}}{\text{volume}}
\]

\[
\text{Bulk density} = \frac{5\text{gm}}{\text{bulk volume}}
\]

\[
\text{Tapped density} = \frac{5\text{gm}}{\text{tapped volume}}
\]

Carr’s Index (% Compressibility)

Flow ability of the formulation can be determined by using following equation

\[
\text{Carr’s Index} = \left( \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \right) \times 100
\]

Hausner’s Ratio

It is calculated by using formula

\[
\text{Hausner’s Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}
\]

Angle of repose

Angle of repose has been defined as the acute angle possible between the surface of pile and horizontal plane. The angle of repose was determined by the funnel method. The Solid Dispersion was allowed to flow out of the funnel orifice on a plane Paper kept on the horizontal surface. This forms a pile on the paper. The angle of repose was calculated by substituting the values of the base radius ‘r’ and pile height ‘h’ in the following equation:

\[
\tan \theta = \frac{h}{r}
\]

Hence, \( \theta = \tan^{-1} \frac{h}{r} \)

Where, \( \theta \) = angle of repose

\( h \) = height of the cone

\( r \) = radius of the cone base

2.4 Characterization of Solid Dispersion

2.4.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The prepared Solid Dispersions were characterized by Fourier transformed infrared spectroscopic analysis (FT-IR), to ascertain for any interaction between the drug and the polymers used and to confirm the encapsulation of the drug within polymer matrix. For this purpose, spectra of the pure drug, pure polymer and formulation containing the drug and polymer was taken. An FT-IR spectrometer was used for the analysis in the frequency range between 4000 to 400 cm\(^{-1}\). One mg of the substance was triturated with 300 mg of dry, finely powdered potassium bromide IR, quantity suitable for a disc 13mm diameter. The mixture was grinded thoroughly, uniformly spread in a suitable die and compressed under vacuum at a pressure of about 800 Mpa. The resultant disc was mounted on the holder in the spectrophotometer. A disc was rejected if the visual inspection shows lack of uniformity or if the transmittance at about 2000cm\(^{-1}\) (5µm) or specific absorption band less than 75% without compensation.

2.4.2 In-vitro Dissolution study of Formulation\(^8,13,14,15\)

Dissolution was taken according to lotter et. al using type-II apparatus of USP (DBK instruments, Mumbai) in 7.4 pH phosphate buffer (900ml) medium, at 75 rpm, and at 37±0.5°C. All formulation equivalent to 30mg of Indomethacin was placed in dissolution media and 5ml of sample was withdrawn at time (min)
5, 10,15,20,30 respectively and diluted up to 50ml and then analyzed with double beam spectro photometer. Cumulative drug releases was determined and were compared with the dissolution of pure drug.

2.5 Formulation of 300mg Indomethacin Tablet:
Prepared Solid Dispersion equivalent to 30mg of Indomethacin, was mixed with Magnesium stearate (as lubricant) starch (as binder), Talc (as glidant) and lactose (as diluent) directly compressible excipients and triturated for 15 to 20 minutes. The prepared mixture was then passed through a sieve no 80. The prepared mixture was then compressed by using RIMEK multiple station punching machine. The proportion of various components used in the formulation is given in table 2.

<table>
<thead>
<tr>
<th>Batch/Ingredients</th>
<th>Solid Dispersion (mg)</th>
<th>Starch (mg)</th>
<th>Lactose (mg)</th>
<th>Talc (mg)</th>
<th>Magnesium Stearate (mg)</th>
<th>Total Qty (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>60</td>
<td>50</td>
<td>180</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>B2</td>
<td>120</td>
<td>50</td>
<td>120</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>B3</td>
<td>180</td>
<td>50</td>
<td>60</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>B4</td>
<td>60</td>
<td>50</td>
<td>180</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>B5</td>
<td>120</td>
<td>50</td>
<td>120</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>B6</td>
<td>180</td>
<td>50</td>
<td>60</td>
<td>5</td>
<td>5</td>
<td>300</td>
</tr>
</tbody>
</table>

2.6 Evaluation of Indomethacin Tablet
2.6.1 Weight Variation
The weight variation test was run by weighing 20 tablets individually, calculating the average weight, and comparing the individual tablet weight to the average. The tablets meet the USP test if no more than 2 tablets are the percentage limit and if no tablet differs by more than 2 times the percentage limit.

2.6.2 Friability
The laboratory friability tester is known as the Roche friabilator. Friabilator subjects a number of tablets to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, dropping the tablets a distance of six inches with each revolution. Normally, a pre-weighed tablet sample is placed in the friabilator, which was then operated for 100 revolutions. The tablets are then dusted and reweighed. Conventional compressed tablets that lose less than 0.5 to 1.0% of their weight are generally considered acceptable.

2.6.3 Hardness
Tablet requires certain amount of strength or hardness to withstand mechanical shock of handling in manufacture, packaging and shipping. The hardness of the tablet was measured by Monsanto hardness tester. The tester consists of a barrel containing a compressible spring held between two plungers. The lower plunger is placed in contact with the tablet and a zero reading was taken. The upper plunger is then forced against a spring by turning threaded bolt until the tablet fractures.

2.6.4 Thickness
Thicknesses of the 10 tablets were measure by using Screw-gauze.

2.6.5 Disintegration test
The disintegration time was calculated by using Tablet Disintegration Tester, at 37ºc ± 2ºc, containing 1L of pH 7.4 phosphate buffers as disintegrating medium.

2.6.6 Drug content
30mg of Indomethacin containing formulation (300mg) dissolved in 7.4 pH phosphate buffer to produced 100ml of solution. 10ml of solution was then diluted to 100ml, by using phosphate buffer of pH 7.4 and then analyzed in Double beam spectrophotometer at 320 nm.

2.7 In-vitro Dissolution of Formulation of tablet
Dissolution was taken according to lotter et.al using paddle type-II apparatus of USP, (DBK instruments, Mumbai) in 7.4 pH phosphate buffer (900ml) medium, at 75 rpm, and at 37±0.5ºc. 300mg of Indomethacin tablet was placed in dissolution media and 5ml of sample was withdrawn at time(min) 5,10,15,20,25,30

© International Journal of Pharma Research and Health Sciences. All rights reserved
respectively and diluted up to 50ml (DF=10) and then analyzed with double beam spectro photometer.

3. RESULTS AND DISCUSSION

3.1 Determination of Drug content
Drug content was determined by using equation prepared from the Calibration curve of the Indomethacin in 7.4 pH phosphate buffer. Drug content in Solid Dispersion prepared using PEG-4000& PVP K30 (Figure 1) and calculated by using equation (Y=0.021X+0.001). Formulation containing PEG 4000(1:5) shows highest percentage of drug content i.e. 99.78% as compared to other formulations.

Fig 1: Percentage Drug content for PEG-4000 & PVP K30

3.2 Micromeritic Evaluation of Solid Dispersion
The Micromeritic property of solid dispersions were characterized with respect to the bulk density, tap density and Carr’s index, hausner’s ratio & angle of repose (Table 3).

<table>
<thead>
<tr>
<th>Batches/Parameters</th>
<th>PEG-4000</th>
<th>PVP K30</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.55±0.03</td>
<td>0.68±0.02</td>
</tr>
<tr>
<td>B2</td>
<td>0.51±0.04</td>
<td>0.64±0.02</td>
</tr>
<tr>
<td>B3</td>
<td>0.5±0.04</td>
<td>0.62±0.01</td>
</tr>
<tr>
<td>B4</td>
<td>0.5±0.04</td>
<td>0.6±0.01</td>
</tr>
<tr>
<td>B5</td>
<td>0.52±0.03</td>
<td>0.6±0.05</td>
</tr>
<tr>
<td>B6</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The values obtained lies within the acceptable range and with no much difference found between loose bulk density and tapped bulk density. These results may further influence property such as compressibility and tablet dissolution. The percent compressibility of powder mixture was determined by Carr’s compressibility index the shown in Table 3. All the formulation so good compressibility. Hausner’s ratio was found to be in a range of 1.17 to 1.25 which shows good flow property. The result of angle of repose of all the formulations. The values were found to be in range of 27° to 31° indicating good flow properties and this was further supported by lower compressibility index values.

3.3 Characterization of Solid Dispersion

IR Spectroscopy
The prepared Solid Dispersions were characterized by Fourier transformed infrared spectroscopic analysis (FT-IR) and shown in figure 2, 3 & 4.

Fig 2: IR Spectra of Indomethacin
Fig 3: IR Spectra of formulation with PEG 4000 + Indomethacin
Fig 4: IR Spectra of formulation with PVP K30 + Indomethacin

Indomethacin and one of the formulations of each polymer were subjected to FT-IR spectroscopic analysis, to ascertain whether there is any interaction between the drugs and the polymers used. From the data obtained it was observed that characteristic absorption peaks appeared at frequencies 451.36 and 464.86 cm⁻¹ (C=O stretch, acid and aromatic), 669.32 (C-H deformation), 835.12 and 752.19 cm⁻¹ (C-H out of plane deformation) 1018.45 and 1102.34 cm⁻¹ (C-O stretch of primary alcohol), 1228.57 cm⁻¹ (C-O stretch plus O-H deformation), 1454.23 cm⁻¹ (O-CH₃ deformation), 1593.09 cm⁻¹ (aromatic C=C stretch),
1716.53 and 1691.46 cm\(^{-1}\) (C=O stretch) and 2966.31 and 2831.46 cm\(^{-1}\) (aromatic C-H stretch and carboxylic acid O-H stretch) for Indomethacin. The peaks obtained in the spectra’s of each formulation correlates with the peaks of drug spectrum, indicating that the drug was compatible with formulation and other additives used in the preparation of the solid dispersion. The characteristic peaks of the pure drug were compared with peaks obtained from their respective formulations and are given in the figures respectively.

### 3.4 In vitro dissolution study of Solid Dispersion

Dissolution Cumulative drug release was determined and was compared with the dissolution of pure drug and shown in figure 5. All Solid Dispersion Formulations satisfies the dissolution acceptance level \((Q_{\text{min}}=75\%\)), where PEG (1:5) batch shows better resistance to drug degradation in dissolution medium pH 7.4 phosphate buffer. Up to 78% of drug was available in dissolution media for the absorption after 40 min. From the dissolution data it can be concluded that batch PEG (1:5) can satisfy our objective of overcoming the variable bioavailability of Indomethacin. The Percentage cumulative drug release was compared with the pure drug; it shows that the solubility is increases when it is formulated with solid dispersion.

![Fig 5: in vitro dissolution study of Solid Dispersion](Image)

### 3.5 Evaluation of Indomethacin Tablets

The Evaluation of Indomethacin Tablet was characterized with respect to the Hardness, Percent friability, Content uniformity, Weight variation, Thickness & Disintegration Time (Table 4). Tablet shows good release, up to 86.98 % of the drug was release to the dissolution media within 40 minute. For the immediate release tablet dissolution acceptance level \((Q_{\text{min}}=75\%)\) which was satisfied. The hardness of tablet was remained between 3.8-4.3 and the %friability was less than 0.7% indicating that it can resist the transportation stress. The drug content of the tablets was found in between the 99.41 % and The Disintegration times of all batches are fewer than 15 mins which passes the USP Limits.

### Table 4: Evaluation of Indomethacin Tablets

<table>
<thead>
<tr>
<th>Batches</th>
<th>Hardness (kg/cm(^2))</th>
<th>Percent friability</th>
<th>Content uniformity (%)</th>
<th>Weight variation (mm)</th>
<th>Thickness (mm)</th>
<th>Disintegration Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>4.3±0.3</td>
<td>0.6±0.02</td>
<td>99.23</td>
<td>passes</td>
<td>3.37±0.03</td>
<td>7±0.08</td>
</tr>
<tr>
<td>B2</td>
<td>4.1±0.2</td>
<td>0.5±0.03</td>
<td>96.16</td>
<td>passes</td>
<td>3.28±0.06</td>
<td>12±0.04</td>
</tr>
<tr>
<td>B3</td>
<td>4.2±0.2</td>
<td>0.5±0.03</td>
<td>99.41</td>
<td>passes</td>
<td>3.29±0.02</td>
<td>14±0.03</td>
</tr>
<tr>
<td>B4</td>
<td>3.8±0.3</td>
<td>0.57±0.04</td>
<td>98.37</td>
<td>passes</td>
<td>3.27±0.02</td>
<td>12±0.05</td>
</tr>
<tr>
<td>B5</td>
<td>4.3±0.4</td>
<td>0.67±0.01</td>
<td>97.13</td>
<td>passes</td>
<td>3.26±0.03</td>
<td>13±0.08</td>
</tr>
<tr>
<td>B6</td>
<td>4.2±0.5</td>
<td>0.61±0.04</td>
<td>99.12</td>
<td>passes</td>
<td>3.23±0.03</td>
<td>17±0.03</td>
</tr>
</tbody>
</table>

### 3.6 In vitro dissolution study of Indomethacin tablets

Dissolution was taken according to lotter et.al using paddle type-II apparatus of USP, (DBK instruments, Mumbai) in 7.4 pH phosphate buffer (900ml) medium, at 75 rpm, and at 37±0.5ºc. 300mg of Indomethacin tablet was placed in dissolution media and 5ml of sample was withdrawn at time(min) 5,10,15,20,25,30 respectively and diluted up to 50ml (DF=10) and then analyzed with double beam spectro photometer. (figure 6)

![Fig 6: In vitro dissolution study of Indomethacin tablets](Image)
4. CONCLUSION
Observations of all formulations for physical characterization had shown that, all of them comply with the specifications of official pharmacopoeia and/or standard reference. Results of in vitro dissolution indicated that all bathes shows cumulative drug release 75% in 30 mins. Results of drug content and in vitro dissolution study indicated that that batch PEG (1:5) was having considerable content uniformity and desirable dissolution. The tablets of that batch PEG (1:5) had considerable content uniformity with good drug release pattern. Thus From the discussion it was concluded that PEG increases the solubility of Indomethacin as compared to PVP K30 at 1:5 drugs to polymer ratio.

5. REFERENCES