DEVELOPMENT OF ABELMASCHUS ESCULENTUS L BASED NANOPARTICLES FOR PERMEABILITY ENHANCEMENT OF RIZATRIPTAN BENZOATE VIA NASOPULMONARY ROUTE

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***Abstract*- Rizatriptan benzoate (RB), a selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist has only 40–50% bioavailability orally presystemic metabolized by the enzyme monoamine oxidase-A (MAO-A). This bioavailability issue can be solved with improved AUC brain ratios through the development of nose-to-brain delivery systems. Nose-to-brain delivery of RB nanoparticles (NP) is investigated to enhance its bioavailability through avoidance of the presystemic first-pass effect. Polymeric NP from Abelmaschus esculentus L (AE) was prepared by crosslinking method. NP possesses size range of 50-300 nm for RB. The pH was found to be 5.99±0.54 results a polysaccharide solution within the physiological range, hence it is non-irritating to the biological mucosa. Previously surface tension and swelling index demonstrated its mucoadhesive uses. The natural polysaccharide derived from AE can be utilized for the fabrication of NP using the emulsification method by using distilled water, drug, castor oil, and Span 80 (5% v/v). The thermal behavior, particle size (nm), shape and surface morphology, drug encapsulation effectiveness, drug loading, and *ex-vivo* permeation studies of NP were all characterized. Finally, the *in-vivo* biodistribution of RB in the brain was investigated using the brain homogenization method by adopting the HPLC method to estimate the drug in male Wistar albino rats. Drug-loaded NP DSC thermograms show that all drug molecules have been evenly distributed throughout the polymer matrix. The mean particle size of NP ranges between 256 and 826 nm and increasing the concentration of polymer resultantly increased the particle size of NP. The formulation's SEM analyses were spherical with variable surface roughness. *Ex-vivo* permeation studies show a control release profile for RB improved for intranasal delivery. It concludes that Abelmaschus esculentus L polysaccharide and RB can be implemented in the preparation of NP for nose-to-brain delivery.**

Keywords: **Rizatriptan benzoate, Abelmaschus esculentus L, Nanoparticles, Permeability enhancement, Nasal delivery.**

Abbreviation: Rizatriptan benzoate (RB); monoamine oxidase-A (MAO-A); nanoparticles (NP); Abelmaschus esculentus L (AE); 5-hydroxytryptamine (5-HT1); solid lipid nanoparticles (SLNs); Blood-brain barrier (BBB); Differential Scanning Calorimetry (DSC); scanning electron microscopy (SEM); Drug-Loading Capacity (DL); Encapsulation Efficiency (EE); Area under curve (AUC).

I. Introduction

Nasopulmonary route is the fastest route of delivery of drug directly to CNS by surpassing first pass metabolism. But there is a problem with this route which is mucoadhesion inside the nasal cavity and the BBB, which is made up of the endothelium of the capillaries in the vertebrate brain, makes it difficult to deliver drugs to the brain [19]. The BBB and the microvasculature in the human brain provide high resistance to the entry of therapeutic agents and other substances into the brain [16]. Several tools were suggested to improve the drug efficacy in CNS through nasal route such as Nanotechnology-based targeting device of polymeric surfactant has great significance in the study of tiny structures, 1 nm to 100 nm in size, through the application of nanotechnology. High permeability, reactivity, surface area, and quantum properties are just a few of the benefits that NP offers [17]. Passive and active transport are the two categories into which the mechanisms of NP crossing BBB can be divided [18]. NP, liposomes, permeation enhancers, mucoadhesive formulations, etc. are some developed nano-based systems for the drugs including biologics are encapsulated into particulate vectors bounded with a particular carrier system (such as synthetic NP). Particulate dispersions or solid particles with a size between 10 and 1000 nm are referred to as NP. Understanding the nasal physiology concerning the olfactory region and trigeminal nerve, which are both involved in the transportation of drugs through the BBB, is necessary to comprehend the delivery of NP to the CNS from the nasal cavity [20]. Polymer based drug delivery system suggested the enhancement of mucoadhesive property of drug [21].

AE also known as okra, is a member of the Malvaceae family and is typically eaten for its immature fresh or dried pods. It is also a versatile species and a multipurpose crop because plant tissues contain a variety of chemical compounds that are used in the food and pharmaceutical industries as well as other less well-known industrial uses (e.g. making biofuel, blood plasma replacement, and stabilizing foams) [10]. Okra's superior fiber slows down how quickly sugar is absorbed from the intestinal tract, which helps to stabilize blood sugar levels [11]. Okra is the best energetic vegetable. Irritable bowel syndrome, sore throat, and lung inflammation are all treated with okra [12]. Okra is thought to prevent the growth of some cancers, particularly colorectal cancer. Diabetes can

be prevented by eating okra [13]. Using a modified rotational cylinder method on animal mucosa, various gum extraction techniques were used to determine the mucoadhesive strengths of AE and Irvingiagabonensis (IG) gums was evaluated and *in-vitro* release of vaccine antigen in the vaccine-gum formulations [14]. Additionally, okra gum rather than pre-gelatinized starch was reported to be an effective binder for naproxen sodium tablets [15].

RB is a 5-HT1 receptor agonist that specifically binds to the serotonin 1 and ID receptors in intracranial arteries to cause vasoconstriction. It is primarily medicated on migraine [1-2]. The oral bioavailability of RB is 40-45% as it undergoes extensive first-pass metabolism. RB's solubility was tested by dissolving it in various solvents and visualizing the results. This demonstrated that the medication was entirely soluble in water, only slightly (by 95%) soluble in ethanol, and completely insoluble in ether and chloroform. These results suggested the actual solubility factors of the RB which also indicates its purity [3]. There are various formulations developed for RB.

Mouth-dissolving tablets were also evaluated for RB [4].To improve drug absorption when administered via the nasal route and increase the drug's bioavailability, SLNs of RB were reported prepared by using the modified solvent diffusion method. These particles are safe and sustained release nasal delivery systems for the treatment of migraines [5]. The use of okra polysaccharide as a carrier for the creation of mucoadhesive delivery systems for nasal drug delivery is supported by mucoadhesive microsphere using AE polysaccharide as a novel carrier for safe and efficient delivery of RB [6]. The ability of RB-SLNs to treat migraines has improved [7].

Additionally, Real-world blood serum samples and RB determination in pharmaceuticals have both been successfully tested using a sensitive and selective electrochemical sensor for RB determination [8]. Hybrid multifunctional lipid-coated graphene oxide mesoporous silica nanocomposite has previously reported RB-controlled delivery. The fabricated carrier for the targeted delivery of RB for the treatment of migraines exhibits longer circulation time due to lipid delivery [9]. Polymers are used additionally for lipid delivery.

Table A: A comparative study of various formulations for RB encapsulation

S.No.	Nano-carrier designed	Purpose	Reference
1.	Mouth-dissolving tablets	Extended Release	[4]
2.	Solid lipid nanoparticles	Nasal delivery	[5]
3.	Microspheres with AE	Nasal delivery	[6]
4.	RB-SLNs	Migraine treatment	[7]
5.	Nanocomposites of RB	Controlled delivery	[9]

To overcome constitutional inconvenience related to conventional dosage forms of RB an alternative drug delivery system in the form of natural polysaccharide-based NP was developed which possesses easy scale-up. The aspect of the study was to explore the ex-vivo permeation effect of NP developed from AE and RB loading. It is also intended to investigate the *ex-vivo* permeation effect enhancement.

II. MATERIALS & METHODS

Cipla (Raighar, India) gave us RB as a kind of gift sample. In Meerut Institute of Engineering and Technology, Uttar Pradesh, India, AE was raised. All other chemicals and reagents used in this study were of analytical quality. Both the experiments and the sample dilutions were done by double-distilled water.

1. Isolation of polysaccharide from AE Fruits

To extract the polysaccharide, immature fruits were used since they contain a higher level of mucilage than matured fruits. The sliced AE fruits were allowed to dip in the required quantity of distilled water for 12 h for the proper extraction. Eight folds of muslin cloth were used to filter the greenish viscous solution. To precipitate the polysaccharide, acetone was added in three volumes to the greenish viscous solution. The pale white polysaccharide was air dried, powdered, and sieved no. 60 before being stored in desiccators until further use [6].

2. Characterization of isolated AE polysaccharide

2.1. Organoleptic Evaluation

The isolated polysaccharide's organoleptic qualities, including colour, flavour, aroma, fracture, and texture, were evaluated [22].

2.2. pH of Polymer solution

A 1 % weight-to-volume solution of powdered polysaccharide was prepared by weighing and dissolving it in water separately. A digital pH meter was used to measure the pH of the solution [22].

2.3. Surface Tension

The drop count method, which is described in Quality control methods for materials of medicinal plant, was used to assess the surface tension of the isolated polysaccharide using a stalagmometer [22].

2.4. Swelling index of polysaccharide

The volume (ml) absorbed by swelling of 1 g of test material under specific conditions is known as the AE polysaccharide swelling index (also known as the swelling capacity). The swelling indications of the selected polysaccharide were discovered using the techniques described in Quality control procedures for medicinal plant materials.

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hours. The volume of mucilage was then calculated, taking into account any sticky mucilaginous components. Three times the process was carried out, and each time the mean value was calculated. Following are the measurements and calculations for swollen volume:

Swollen volume (V2-V1), where V1 and V2 are the initial volumes of the material before hydration and the volume of the hydrated material, respectively [6].

2.5. Measurement of Mucoadhesive Strength of AE Polysaccharide

Using a force measurement, the mucoadhesive potential of polysaccharides was evaluated. The membrane of a goat's nose was removed carefully from a nearby slaughterhouse. The surface area of each exposed nasal mucosal membrane was 1 cm^2. A predetermined number of samples of each formulation were placed on the lower probe at room temperature. The probes were maintained at 37+2°C and were calibrated. Nasal tissue was placed inside a probe and lowered until it made contact with the sample's surface [24]. A force of 0.1 N was immediately applied for 2 minutes to guarantee close contact between the tissues and the samples. The probe was then raised at a constant rate of 0.15 mm/s. According to the formula in Quality control methods for medicinal plant materials, the mucoadhesive force was calculated from the minimal weights that allowed tissues to separate from the surface of each formulation.

Detachment stress = MG/A

M = weight in grams added to the balance

G= Gravitational acceleration is assumed to be 980 cm/s2.

A = surface area of nasal mucosal tissue cm^2. A new smooth gel surface was created before each measurement [25].

2.6. Loss on Drying

A glass bottle with a tarred cork was used to weigh 1g of polysaccharide power. The bottle was dried in a hot air oven at 105 °C, and the weight was measured every hour until a steady weight was reached. Utilizing quality control methods for medicinal plant materials was necessary to determine the proportion of weight lost by the powder [26].

2.7. Solubility Studies

Any excipient's solubility behavior can be used to gauge how well it will work with various formulations. The solubility of one part of dry powdered polysaccharide was assessed by shaking it with various solvents such as water, ethanol, methanol, chloroform, n-butanol, hexane, benzene, and ether [27].

2.8. PREFORMULATION STUDIES

Preformulation study is an important tool for determination of physical and chemical properties of the drug before incorporating it into the formulation development. The nature of the drug highly affects the processing like method of preparation, entrapment efficiency, compatibility and pharmacokinetic response of the formulation. Preformulation studies are indispensable protocol for development of safe, effective and stable dosage form. Thus, in order to ensure optimum conditions for clinically beneficial delivery system, preformulation studies were carried

out. A thorough understanding of these properties, ultimately provide a rational for formulation design. A preformulation study includes drug identification test, solubility study, preparation of standard curve and drug excipient compatibility studies. These studies were done in this phase to provide a useful support in development of dosage form.

3. Fourier Transform Infrared (FTIR) Spectroscopy for Characterization

IR spectra are significant records that provide enough details about a compound's structure. Contrary to the UV spectrum, which has a limited number of peaks, this method produces a spectrum with a large number of absorption bands, which can be used to extract structural information.

At a ratio of 1:100, RB was added to the KBr, and the resulting mixture was then put through a pellet press with dies set at a 10 tonnes pressure for one minute. By turning the side value counterclockwise and removing the pellet from the die set, the pressure was released. This pellet was analyzed to estimate the purity of the sample, an IR graph was obtained and interpreted.

It was similar to the formulation's intended ratio of RB to AE polysaccharide. The physical mixture was prepared, placed in a glass vial, sealed, and maintained in a stability chamber for two weeks at a temperature of 40°C + 1°C. After 2 weeks, physical mixture observations were used to assess compatibility. FTIR spectroscopy was also applied to the physical mixture to look for any molecular interactions. KBr was combined with the dried physical mixture for use in an IR spectrophotometer. An IR spectrum between 400 and 400 cm was recorded following the preparation of the combination and the formation of pellets in the KBr press. To check for any potential interactions, the IR spectra of the physical combination were compared to the spectra of the pure drug and polymer [6].

4. Emulsion cross linking method

The emulsion cross-linking method is one of the preparation techniques frequently used for the preparation of NP. RB is highly water soluble and insoluble in organic solvents, in contrast to AE polysaccharide, which expands in water (ethanol, methanol, isopropyl alcohol). The polar phase (representing the aqueous phase) in the current study was distilled water.

Soybean oil, olive oil, groundnut oil, castor oil, and liquid paraffin are all used to make NP. Due to its accessibility and desired viscosity, a 1:1 mixture of castor oil and octanol was chosen as the external phase for the preparation of NP.

The solubility of drugs and polymers determines the use of solvents. RB is a drug that is highly water soluble but insoluble in methanol, ethanol, and other organic solvents. Therefore, distilled water was used as the internal phase in the current investigation. Tween 80 and Span 80 are frequently used in the production of NP. A dispersing agent was chosen based on solubility criteria in the external phase.

Different solvents including acetone, hexane, diethyl ether, and petroleum ether to wash NP were also used [6].

4.1 Preparation of NP

Different formulations of drug-loaded AE-based NP were produced using the emulsion cross-linking technique [6]. The polysaccharide was disseminated in a specified quantity of distilled water containing the drug to form a solution of polysaccharides containing varying concentrations (0.01% w/v, 0.02% w/v, 0.03% w/v and 0.04%w/v) and stirred gently for 4 h continuously to form a homogenous aqueous solution. The aqueous solution was poured slowly in a mixture of castor oil and octanol (1:1) containing Span 80 (5% v/v) to form a stable emulsion using a mechanical stirrer (Remi) at a rotational speed (15,000 rpm). After 15 minutes, 0.2 mL (or 4-6 drops) of concentrated sulphuric acid and epichlorohydrin (2.5 % v/v) as a cross-linking agent was added to the dispersion, then by stirring at a continuous speed for 18 h. During the process, a constant temperature of 40°c was maintained. NP was separated by centrifugation and removed using several isopropyl alcohol fractions. In table 7.1, the various formulations (F1–F4) are compiled.

4.2 Characterization of NP

4.2.1 DSC Study

Using DSC technology, a typical differential scanning calorimetric examination was conducted to ascertain the thermal behavior of materials. (Mettler, Toledo, DSC 822C, Switzerland) (pure drug RB, AE polysaccharide, and NP). On a crimped aluminum plate, each sample was put, and it was heated at a rate of 10°C/min while being periodically purged with nitrogen (50 mL) [16].

4.2.2 Particle size analysis

The geometric particle size of NP was determined using a laser particle size analyzer (Helos BF, Symantec, Germany). The volume surface diameter of each produced NP particle was utilised to calculate its average size. The prepared NP was suspended in silicon oil (nm) [6].

4.2.3 Shape and surface Morphology

The shape and surface characteristics of NP of all formulations (F1 to F4) used SEM [JEOL Model JSM 6390LV] to assess, Cochin University of Science and Technology Cochin, Kerala, India Sophisticated Analytical Instruments Facility (SAIF)]. The samples were prepared for SEM by being lightly dusted with NP and taped to an aluminum stub. The stubs were then examined in a microscope with a scanning electron detector and sample photos were taken after being coated with silver using a sputtering coater to a thickness of 300 A [16].

4.2.4 Drug-Loading Capacity and Encapsulation Efficiency

The maximum amount of medication that can be incorporated into the NP is known as the loading capacity. Encapsulation efficiency measures how much additional medication is contained within NP formulations [28]. The ratio of the drug in the final formulation to the amount of extra drug was used to calculate encapsulation efficiency [29].

To fully extract the drug from the NP, a measured amount (25 mg) of the drug-encapsulated NP was stirred magnetically for 12 hours at room temperature in 25 ml of a phosphate buffer solution with a pH of 6.8 [30]. The solution was centrifuged at 4000 rpm for 10 minutes to ascertain the amount of rizatriptan benzoate in the mixture. The supernatant was diluted with a phosphate buffer solution of pH 6.8 before being measured at 225 nm with a UV spectrophotometer. Every sample was tested three times, and the results were averaged.

a) This is how EE was calculated:

$$EE\ (\%) = ED \times \frac{100}{AD}$$

Where AD amount of drug added, EE= Encapsulation efficiency; ED = Amount of encapsulated drug.

b) DL was determined as follows:

$$DL\ (\%) = \frac{WD \times 100}{WT}$$

Where DL = Drug loading; WD= Weight of drug loaded in NP; WT = Total weight of NP [16].

4.2.5 *Ex-Vivo* Permeation Studies

Studies of *ex vivo* permeation were conducted using an efficient dissolution method. Using goat nasal mucosa (which was purchased from local slaughterhouse) as the permeation membrane and phosphate buffer (pH 6.8) as the dissolution medium [6]. The open end of the test tube was covered with a single layer of fresh goat nasal mucosa with the help of a thread. The other end of the test tube was broken to open the end for sample introduction. Phosphate buffer (pH 6.8) was used in a 100 ml, and 50 ml beaker, and a magnetic stirrer was used to maintain the temperature at 37 °C. To hold the goat nasal mucosa that had been dipped in the phosphate buffer, the test tube was turned upside down and clamped with a stand. The mucosa layer was the only part that could dip. The surface of the goat nasal mucosa was placed inside the inverted test tube with an accurately weighed quantity of NP equal to 10 mg of RB. The stirrer's speed was then held at 50 rpm. At 30-minute intervals, samples (1 mL) were taken out and replaced with fresh, 6.8-pH phosphate buffer solution in the same volume at the same temperature. Samples were diluted up to 10 ml. and filtered using Whatman filter paper and analyzed spectrophotometrically for RB at 225 nm [16].

III. RESULTS AND DISCUSSION

The yield of the isolated polysaccharide from AE fruits was 8.39% w/w. The isolated polysaccharide was then used for the identification test and physical characterization for formulation development.

5. Physical characterization

5.1. Organoleptic characterization

The organoleptic properties of the AE polysaccharide were off-white with a rough fracture surface texture. It was found to be odorless and have a characteristic taste.

5.2 pH of polysaccharide solution

The pH of the AE polysaccharide solution was examined to determine whether the biological mucosa would accept it. The polysaccharide solution's pH value is 5.99 ±0.54, within the physiological range, and is therefore not irritating to the biological mucosa.

5.3 Surface tension

The result of surface tension study for AE was found 38.1256 ± 4.65N/m.

5.4 Swelling index

To determine the polysaccharide's ability to hold water, the swelling index of an isolated polysaccharide was examined. According to the findings which were 340 ±12% (v/v), AE polysaccharides can be used as a mucoadhesive agent.

5.5 Mucoadhesive strength

The results found were 3.626 ± 0.125 dyne/cm^2, indicating that the AE polysaccharide has ideal mucoadhesive strength and will hold the formulation in the nasal cavity for an extended period, enabling a prolonged release of the medication at the desired site inside the nose.

5.6 Loss on drying

On applying heat, the Loss on drying of AE was 0.1983% (w/w).

5.7 Solubility studies

Any excipient's solubility behavior can be used to gauge how well it will work with various formulations. The solubility behavior was also investigated using isolated polysaccharides. In contrast, organic solvents such as ethanol, methanol, chloroform, n-butanol, hexane, benzene, and ether, completely render the polysaccharide insoluble. When polysaccharide is in contact with water, it expands to create a gel.

6.1. FTIR Spectroscopy

The IR spectra of pure RB were recorded using FTIR spectroscopy, and this data was compared with the functional group frequencies of RB [3]. FTIR tracings and caliberated curve are shown in Figure 1-3, and Table 1 provides the interpretation. The comparison demonstrates unequivocally that the medication is pure RB and contains barely detectable amounts of other substances or impurities.

Drug Excipients Compatibility was studied by Physical changes watched in the RB and excipient mixture (discoloration and caking). After two weeks, the physical mixture exhibited no signs of discoloration or caking. It shows that the drug and excipients work well together. Figure No. 3 displays the physical mixture's IR spectrum. The IR spectra of the pure drug and polymer and those of the physical combination had no appreciable differences. This suggests that there is no possibility of drug-drug interaction with the other formulation ingredients.

Table 1: Interpretation of FTIR spectrum of Rizatriptan Benzoate

F. Code	Frequency observed (cm^{-1})	Frequency of reference (cm^{-1})	Interpretation
1	1606.59 (Stretching)	1600-1800	C=0
2	1204.46 (Stretching)	1000-1245	C-N
3	3105.18 (Stretching)	3100-3400	N-H
	836.08 (Bending)	660-900	
4	1505.34 (Stretching)	1500-1600	C=C

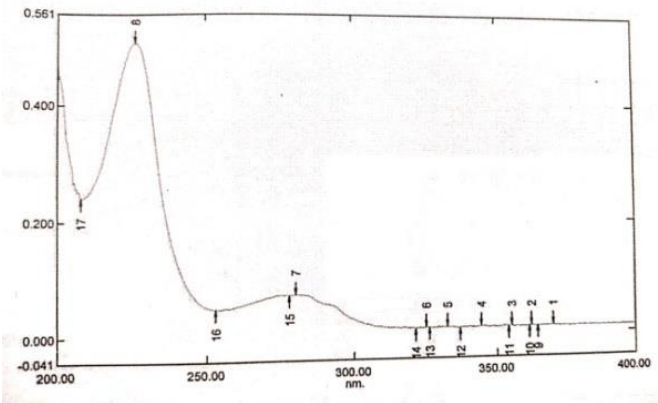


Fig. 1: Rizatriptan benzoate peak in phosphate buffer at 225 nm (pH 6.8)

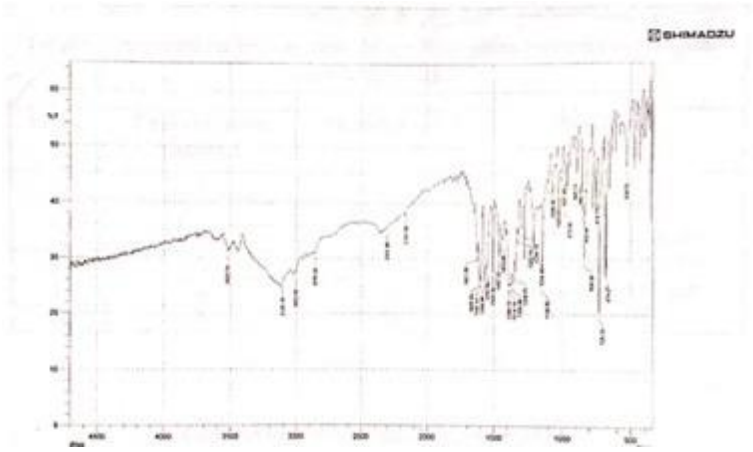


Fig. 2: FTIR spectra of pure Rizatriptan benzoate

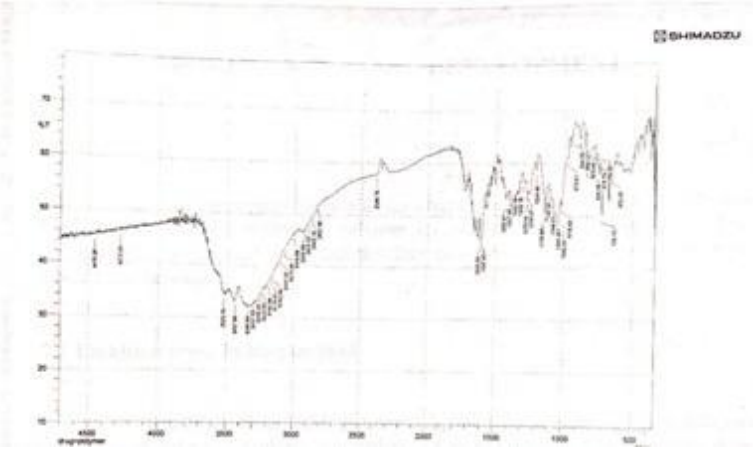


Fig. 3: Rizatriptan benzoate and Abelmoschus esculentus L. polysaccharide physical mixture's FTIR spectra (1:1)

7. Emulsion cross linking method

The current study used the emulsion cross-linking technique to produce NP [35]. The solvent of choice was influenced by the drug and polymer solubilities. While the polysaccharide found in AE swells in water, RB is a drug that is highly water soluble. Therefore, distilled water was used as the internal phase [36].

Castor oil was used as an external phase in the current study, so Span 80—which is soluble in castor oil—was used. AE polysaccharide-based NP was prepared using Span 80 at a 5 percent concentration (v/v). Polysaccharide droplets appear to be protected by Span 80, which also prevents the droplets from coalescing.

Castor oil residue was removed from NP by washing them. It was necessary to choose a washing solvent that was non-solvent for polymer and drug but soluble only in castor oil. Water cannot dissolve castor oil. In contrast, it dissolves in isopropyl alcohol. So, isopropyl alcohol was used as a solvent for washing. The resulting NP was discrete.

7.1. Evaluation of prepared nanoparticles

Without using an emulsion, AE polysaccharide-based NP were created, and they were then chemically cross-linked with epichlorohydrin to harden them. The prepared NP was spherical, free-flowing powder in the required range of size for the nose-to-brain application. The yield of NP in all formulations ranged from 45% to 66% weight to weight.

7.2. Differential Scanning Calorimetry study

Thermal analysis of RB, AE polysaccharide, and prepared NP was determined by DSC. The representative thermo grams of pure drug, AE polysaccharide, and prepared NP are shown in Fig. 4(a), (b), and (c) respectively. The melting point of the medication, 181.99 °C, was visible on the DSC thermogram of the pure substance. The polymer's endothermic transition was evident in the polysaccharide of AE, which had two large peaks at 130.68 and 182.63 °C. Broad peaks were visible in the formulation at 92.63 and 147.84 °C. The polymer completely obscured the drug's peak, which may be the result of the drug molecules being evenly distributed throughout the matrix of the polymer.

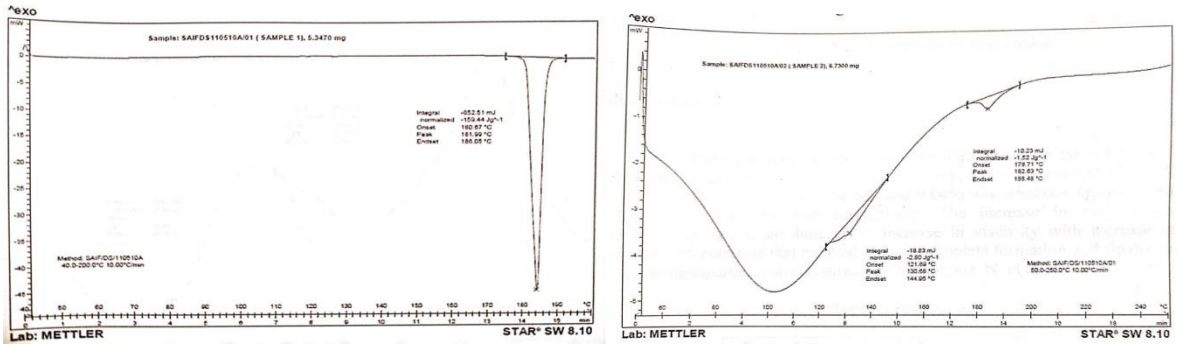


Fig 4(a) Fig 4(b)

Fig. 4(a): DSC of Rizatriptan benzoate 4(b): *Abelmoschus esculentus*L. polysaccharide concentration

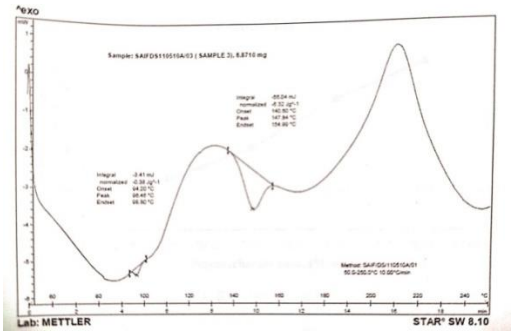


Fig 4(c)

Fig. 4(c): DSC of prepared Nanoparticles

7.3. Particle size

The average size of NP was discovered at concentrations of 0.01 percent (w/v), 0.02 percent (w/v), 0.03 percent (w/v), and 0.04 percent (w/v) of AE polysaccharide as mentioned in Table 2 and figure 5. Bigger droplets led to the production of larger NP, which resulted in an increase in viscosity as the concentration of polymers rose [6].

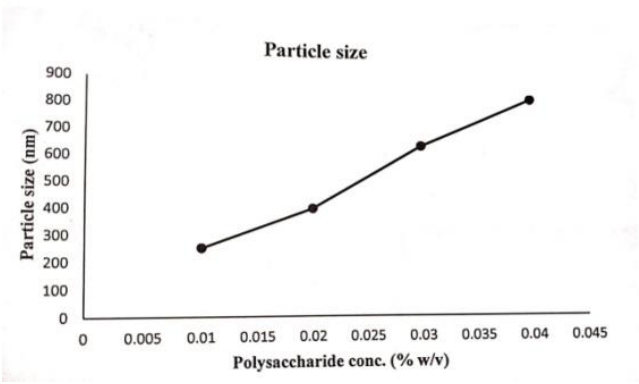


Fig. 5: Particle size

7.4. Surface morphology

The surface morphology and shape of different formulations (F1-F4) of prepared NP at different concentrations of polysaccharide were shown in figures 6(a), (b), (c), and (d). According to visual analysis of SEM images, the NP was spherical with different levels of surface roughness. When polysaccharide was present in the smallest amounts (0.01 % w/v), the particles appeared to be more spherical and had nearly smooth surfaces. The polysaccharide concentration was increased to 0.02 % w/v, 0.03 % w/v, and 0.04 % w/v. As the concentration increased, the particles became less spherical and their surfaces became rougher [6].

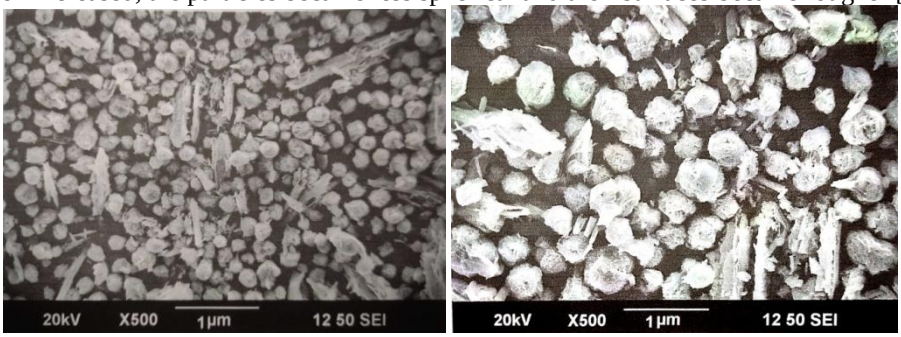
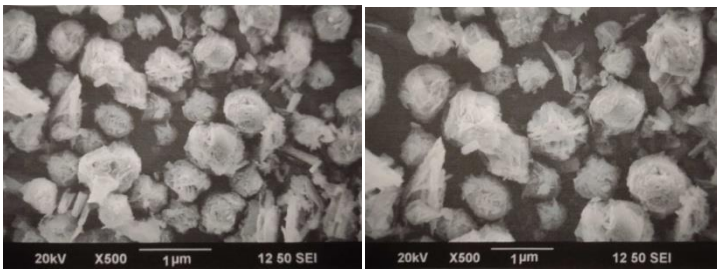


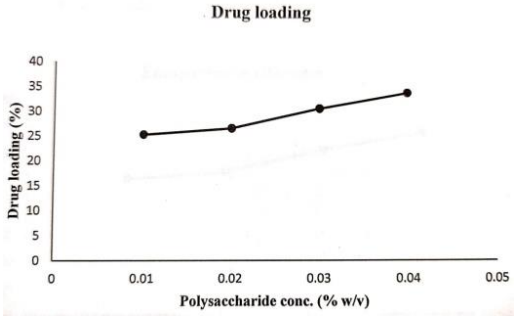
Fig. 6(a): Scanning electron photomicrograph of F1, (b): F2



(c): Scanning electron photomicrograph of F3. (d): Scanning electron photomicrograph of F4.

7.5. Drug loading

The DL of prepared NP at AE polysaccharide concentrations, 0.01% w/v, 0.02% w/v, 0.03% w/v, and 0.04% w/v were found to be 25.35± 2.43%, 26.65±2.61%, 30.7±3.01%, and 33.95±3.34% w/w respectively (shown in Table 2 and figure 7). According to this analysis, DL increases along with polymer concentration. The availability of polysaccharides may reason for increased DL. Because DL depends on the drug-polymer ratio used, Most of the drug remains entrapped if an insufficient amount of carrier polysaccharide is available [6].



7.Fig. 7: Drug loading

5.1. Encapsulation efficiency

The EE of prepared NP at AE polysaccharide concentrations, 0.01% w/v, 0.02% w/v, 0.03% w/v, and 0.04% w/v was found to be 50.7±4.91%, 53.3±5.13%, 61.4±6.02%, and 67.9±6.71% w/w respectively (shown in Table 2 and figure 8). This finding suggests that EE increases along with polymer concentration. The more options there are for using polysaccharides as a drug carrier. The complex network of polysaccharides formed during emulsification at high polysaccharide concentrations prevents drug migration into surrounding media, which could explain the improved value of encapsulation efficiency [6].

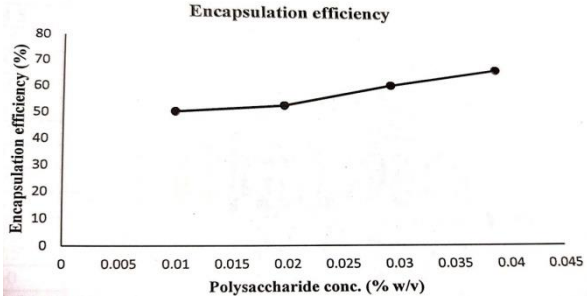


Fig. 8: Encapsulation efficiency

7.5.2. *Ex-Vivo* Permeation Studies

Goat nasal mucosa was used as a permeation membrane in AE *ex-vivo* permeation investigations. Research on polysaccharide-based NP was carried out at 37±1°C in a 6.8 pH phosphate buffer [37]. The study was completed within the time frame necessary for the maximum drug release from the NP. The study clearly shows that the NP had the required control release profile. Drug initial release has been seen to gradually increase over time. Table 2 displays the findings of permeation studies conducted on all formations (F1-F4). Figure 9 depicts the graph between the percentage permeation and time.

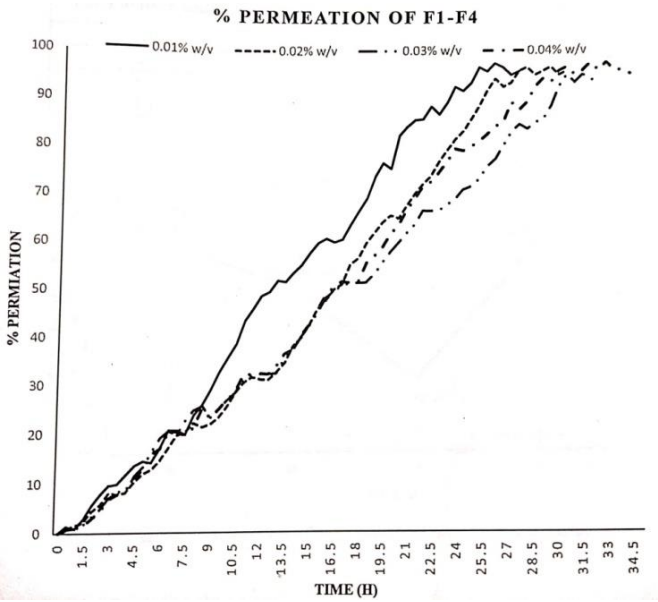


Fig. 9: *Ex-Vivo* Permeation graph of all formulations (F1-F4)

Ex-Vivo Permeation of drug from NP, from the formulations FI (0.01%w/v polysaccharide concentration), F2 (0.02%w/v polysaccharide concentration), F3 (0.03%w/v polysaccharide concentration) and F4 (0.04%w/v polysaccharide concentration) were found to be 97.888±9.7% (after 28 h), 97.029 +9.66% (after 31 h), 95.312±9.47 % (after 33 h) and 97.888± 9.73 % (after 35 h) respectively. With increasing polysaccharide concentration, a difference in the amount of time needed for the maximum permeation was seen in Table 2. The data suggest that with an increase in AE polysaccharide concentration the density of the polymer matrix increases which shows an increase in the diffusion path length that the drug molecule has to travel, consequently, the rate of drug release from the NP decreases.

IV. CONCLUSION

The rationale of the study was to create natural polysaccharide-based nanoparticles (NP) for the nose-to-brain drug delivery system to improve the therapeutic effectiveness and bioavailability of rizatriptan benzoate (RB) in the brain. Thus, the work will contribute to enhancing the efficacy and safety of direct drug delivery from the nose to the brain using biocompatible NP. With a focus on enhancing therapeutic efficacy and relative bioavailability of RB in the brain, the current research work demonstrated the applicability of natural polysaccharide extracted from the fruit of Utilizing Abelmoschus esculentus L. (AE) as a novel carrier and the emulsion cross-linking method, researchers were able to create natural polysaccharide-based NP. Prepared NP has a mean particle size in the 256±75-826±257nm range, which is the size needed to enter the brain through the trigeminal nerve and/or the olfactory epithelium. The range of prepared NP encapsulation efficiency was found to be 50.7±4.9-67.9±6.71 %w/w. The range of the drug loading of the prepared NP was 25.35±2.43-33.95±3.34 %w/w. All the batches of NP were found to release 95.312±9.47-97.888±9.73% drug in 28-35 h which specifies the necessary control release profile of formulation. Thus, we can conclude that the NP of RB could be an approach for nasal to cerebral drug delivery using okra polysaccharide for permeability enhancement.

Table 2: Formulations of Abelmoscina esculentus L. Polysaccharide based drug loaded nanoparticles

F. Code	Sur (%v/v)	CA (%v/v)	DPR	PC (%w/v)	Size (nm)	DL (%w/w)	EE (%w/w)	Time (hour)	MP (%)
F1	5	2.5	1:1	0.01	256±75	25.35±2.43	50.7±4.91	28	97.888±9.7
F2	5	2.5	1:1	0.02	409±139	26.65±2.61	53.3±5.13	31	97.029±9.66
F3	5	2.5	1:1	0.03	651±241	30.7±3.01	61.4±6.02	33	95.312±9.47
F4	5	2.5	1:1	0.04	826±257	33.95±3.34	67.9±6.71	35	97.888±9.73

Sur= Surfactant, CA= Cross linking agent, DPR= - Drug: Polymer ratio, PC= Polysaccharide concentration, Size= Particle Size, nm= Nanometer, DL= Drug Loading, EE= Encapsulation Efficiency, Time= Study time and MP=Maximum Permeation

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