thymifolia (fig.1). The above procedure was followed by taking 0.5 ml of rat brain homogenate with different concentrations (0-1000 µg) of ethanolic extract of *E. thymifolia* (Table 2).

The effect of ethanolic extract of *E. thymifolia* on lipid per oxidation level, both *in vivo* and *in vitro* is summarized in Table 1 and 2. The results obtained indicate that the ethanolic extract of *E. thymifolia* has significantly reduced the free radical induced lipid peroxidation of rat brain phospholipids in both *in vivo* and *in vitro* experiments. In case of *in vivo*, it was found to be comparable to vitamin E, while in *in vitro* study a dose-dependant response at lower doses was observed.

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Modulated Permeation of Insulin through Glucose Sensitive Membrane

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Glucose sensitive membrane was prepared by solution casting method containing pH sensitive microparticles. The membranes consisted of semiinterpenetrating polymer network microparticles of poly(vinyl alcohol) and poly(methacrylic acid), Glucose oxidase and catalase enzyme. The immobilized glucose oxidase acted as the glucose sensor and catalyzer; it was sensitive to glucose and catalyzed the glucose conversion to gluconic acid. Catalase was used to convert hydrogen peroxide to oxygen. There was no detectable leakage of enzymes in the release media and the activity of the immobilized enzymes was 70% compared to that of free enzymes. The permeability of insulin was dependent on the glucose concentration. The insulin permeability increased 3 times when the glucose concentration was increased from 50 mg% to 200 mg% and increased by 7 times when the glucose concentration was 400 mg%.

Diabetes mellitus is a major cause for the death in industrialized countries and multiple parenteral injection of insulin are currently the standard treatment for insulin dependent diabetic patient. A variety of degenerative conditions observed in diabetics such as neuropathy and increased vascular disease may stem from the poor control of the blood glucose level due to subcutaneous injections[1,2]. Of the various delivery techniques developed for insulin delivery, self-regulated insulin delivery systems have attracted growing interest due to the presence of both glucose sensing and insulin delivery functions. The self-

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regulated delivery systems are designed so as to mimic the physiological release of insulin in response to changes in glucose concentration[3,4]. Various sensing mechanisms like pH-dependent polymer erosion[5], substrate enzyme reaction[6], drug solubility[7], competitive binding[8] and various types of devices like hydrogel membranes[c], matrix system[9] and grafted porous membranes[10] are used to develop self-regulated insulin delivery.

We have developed the glucose sensitive membrane containing pH sensitive microparticles of poly(vinyl alcohol) and poly(methacrylic acid) semiinterpenetrating polymer network. The microparticles were dispersed in the ethyl cellulose membrane along with glucose oxidase and catalase enzymes. The permeability of insulin across the membrane increases with decreasing pH of the external solution. The microparticles remain swollen at normal blood pH and when the glucose concentration increases, the pH is lowered due to the formation of gluconic acid catalyzed by glucose oxidase (GOD) and catalase enzymes[9]. This causes the microparticles to shrink allowing the insulin to diffuse through the void created in the membrane.

Methacrylic acid (Aldrich, Steinheim, Germany) was made inhibitor free by distillation, poly(vinyl alcohol) (average molecular weight range 1 24 000–1 86 000 and degree of hydrolysis 99%, Aldrich), *N,N*-methylenebisacrylamide (Sigma, St. Louis, USA), potassium persulphate (KPS, Rankem), ethyl cellulose (10 CPS, Signet), insulin (from porcine pancreas, Sigma, St. Louis, USA), glucose oxidase (from *Aspergillus niger*, Sisco), catalase (HiMedia) and dextrose (Rankem) were used without purification. Double distilled water was used throughout the experiment.

The semiinterpenetrating polymeric networks of varying composition were prepared by the free radical polymerization method. Poly(vinyl alcohol) (1% w/v) was dissolved in water heated to 90° and stirred for 12 h. When the solution attained room temperature, methacrylic acid (15 mM), *N,N*-methylenebisacrylamide (0.02 mM) and potassium persulphate (0.2 mM) were added. The mixture (20 ml) was homogenized and kept at 70° for 4 h so that the entire mass converted into thin white films. The films equilibrated with double distilled water for 96 h to remove the unreacted monomers. The swollen hydrogel was then dried at room temperature for 72 h and weighed. This process was continued till constant weights were obtained. The dried hydrogel was then powdered and passed through sieve #300 and #350. Particles, which passed through sieve #300 but retained on #350, were used for the preparation of glucose sensitive membrane.

Glucose sensitive membrane was prepared by using solution casting method. The microparticles were dispersed in acetone along with glucose oxidase (5 mg) and catalase (1.44 mg). Ethylcellulose (3% w/v) dissolved in acetone was added to this mixture and stirred. The amount of microparticles added was 25% of the total weight of the membrane. The mixture was poured into petridish and kept in dessicator. After evaporation of the solvent a membrane of thickness ~0.015 cm was obtained. The membrane was washed with double distilled water and stored at 4° in buffer (pH 7.4).

The efficiency of enzyme immobilization in the membrane was determined by measuring the amount of unbound enzymes. The amount of the glucose oxidase and catalase in the washing were estimated by spectrophotometer at 450 and 280 nm, respectively. The efficiency of enzyme immobilization was found to be in the range of 86-90%. The activity of glucose oxidase in the membrane was determined by measuring the ability of immobilized enzyme (4.5 mg) to lower the pH of the glucose solution (200 mg) compared to free glucose oxidase (3, 4, and 5 mg). The membrane (0.45 g) containing 25 wt % of

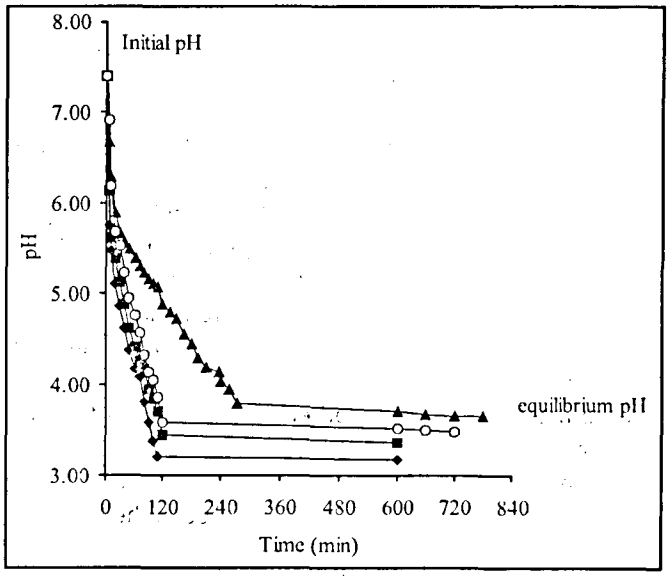

Fig. 1: The activity of free and immobilized glucose oxidase.

pH change for free GOD 3 mg(-▲-), 4 mg (-■-), 5 mg(-◆-) and 4.5 mg of immobilized GOD (-0-). The test solution used was 100 ml saline solution containing 200 mg of glucose (pH 7.4)

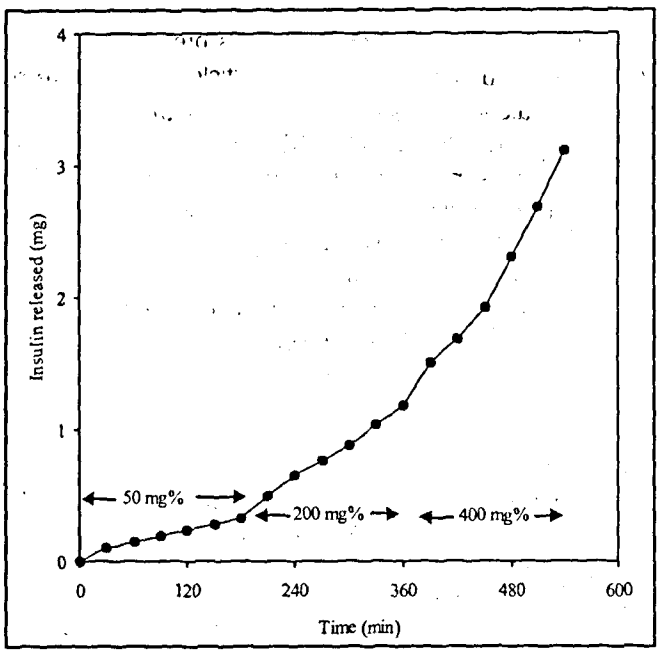

Fig. 2: Insulin permeation across the membrane.

The membrane consisted of 25 wt% of pH sensitive microparticles, the donor compartment was filled with insulin solution (1 mg/ml) and the receptor compartment with 50, 200, 400 mg% of glucose concentration in pH 7.4 phosphate buffer solution at 37°.

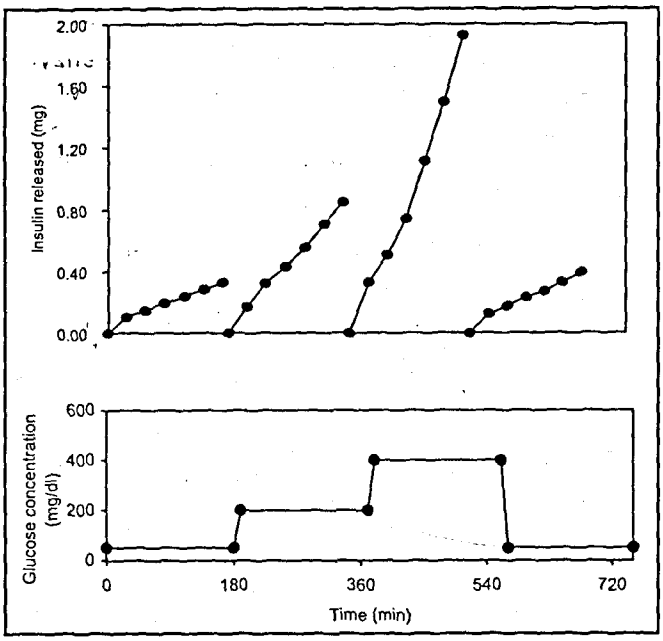

Fig. 3: Insulin delivery across membrane in response to step changes in glucose concentration.

The membrane consisted of 25 wt% of pH sensitive microparticles, the donor compartment was filled with insulin solution (1 mg/ml) and the receptor compartment with 50, 200, 400 mg% of glucose concentration in pH 7.4 phosphate buffer solution at 37°.

microparticles and thickness (0.015 cm) was immersed in 100 ml saline solution containing 200 mg of glucose. The pH of the solution was adjusted to 7.4 using 0.1 N NaOH. The time for the pH to decrease from 7.4 to 4 was 40, 70, 215 min for the sample of 5, 4, 3 mg of free GOD, respectively, and was 120 min for the sample of 5 mg of immobilized GOD (fig. 1). The equilibrium pH of the immobilized GOD was 3.49 and that of free GOD was 3.36 and 3.65 for 4 and 3 mg, respectively. From these data it is clear that the activity of 5 mg immobilized GOD is between 4 and 3 mg of free enzyme. Therefore it can be concluded that the activity of immobilized enzyme is 70% of the free enzyme. The low activity of the immobilized enzyme compared to free enzyme can be attributed to the inaccessibility of some of the reaction sites of the GOD.

Insulin permeability through the membrane was measured using side-by-side diffusion cell. The volume of the cell was 17 ml and exposure area was 0.73 cm^2. The donor cell was filled with insulin solution (1mg/ml) and the receptor cell was filled with 7.4 pH phosphate buffer solution (PBS) with various glucose concentrations (50, 200, 400 mg/dl). The insulin solution was prepared by dissolving insulin in 0.1 N HCl and then diluting with pH 7.4 PBS. The membrane was placed between two cells and the diffusion cell was tightly sealed and then immersed in a water bath. Magnetic bars were placed in each cell to provide mixing. From the donor cell 0.5 ml of sample was removed and insulin was estimated by UV spectrophotometer at 276 nm[11]. Insulin permeability was calculated using equation P=(dc/dt)Vd/CA, where P is permeability, dc/dt is the slope of the straight line obtained by plotting insulin released against time, V and C are the volume of the upstream compartment and the initial concentration of insulin, respectively. A and d are the area and the thickness of the membrane, respectively[12].

The receptor cell was initially filled with 50 mg/dl glucose solution and after 3 h this solution was replaced with 200 mg/dl solution and then with 400 mg/dl. The concentration of insulin in the donor compartment was maintained at 1 mg/ml throughout the experiment. After the first cycle the receptor cell was filled with 50 mg/dl glucose solution for overnight run. The permeability of insulin was determined at different glucose concentration and is shown in fig. 2. Fig. 3 shows insulin release in response to step

changes to glucose concentration. The permeability ratio of insulin (P_{50}:P_{200}:P_{400}) was found to be 1:3:7.4, 1:2.6:7, 1:2.6:6.9 for cycles 1, 2 and 3 respectively. The average P_{50}, P_{200}, P_{400} for three cycles was $1.1x10^{-5}$, $3.06x10^{-5}$ and $7.85x10^{-5}$. It is evident from the data that the insulin release depends on the external glucose concentration and when the glucose concentration was increased from 50 to 200 mg% the insulin release increases by three times and when increased to 400 mg% the release increases by almost 7 times.

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Spectrophotometric Determination of Ambroxol Hydrochloride

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A rapid, sensitive and simple spectrometric method is developed for the estimation of ambroxol hydrochloride. It is based on the reaction of p-N-dimethylamino-cinnamaldehyde reagent with aromatic amino group of ambroxol in acidic medium. The yellow chromophore with λ max at 503 nm, obeyed Beer's law in the concentration range of 1-110 µg/ml.

Ambroxol hydrochloride, an expectorant is useful in the treatment of respiratory problems. The drug is official in BP[1]. Chemically ambroxol hydrochloride is trans-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride. Literature cites determination of ambroxol hydrochloride by spectrophotometric[2], HPLC[3-5] and GC methods[6-7] in pharmaceutical formulations. Officially, ambroxol is assayed by potentiometric titration[1] method. In the present study, the presence of aromatic amino group in ambroxol was exploited, for a condensation reaction with 3-(4'-N,N-dimethylaminophenyl)-prop-2-en-1-al, more commonly referred to as p-N-dimethylamino-cinnamaldehyde reagent (PDAC).

A Shimadzu UV 1601 Spectrophotometer with 1 cm matched quartz cell was used for developing the method. Toluene and methanol of analytical grade were used in the proposed method. A solution of 0.1% w/v PDAC was prepared in methanol. A standard solution of ambroxol hydrochloride was prepared by dissolving 100 mg of ambroxol hydrochloride in methanol and then the volume

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