INTRODUCTION
2. INTRODUCTION

Diabetes is an age-old disorder affecting millions of patients worldwide. It is a metabolic disorder, which is primarily controlled by physical exercise and diet, however these two appear to have significant effect on glycemic control, and they are insufficient to get the glycemic levels to a desired extent. Pharmacotherapeutic measures hence become essential in maintaining near normal glycemic levels in diabetes. The drugs used in diabetes include insulin, sulfonylureas, biguanides, glitizides, thiazolidinediones and alpha glucosidase inhibitors (Edelman & Henry, 1995; Lebovitz, 1992). Treatment with insulin is must for type-1 diabetic patients; however, in patients with type 2 diabetes, the use of insulin is essential when diet, physical exercise and oral antidiabetic agents fail to maintain glycemic levels (American Diabetes Association, 1994; Alberti et al., 1994).

The discovery of insulin by Banting and Best is one of the miraculous achievements of twentieth century medicine (Banting & Best, 1922). Eli lily and company introduced the first commercial insulin preparation in 1923, the first rDNA human insulin nearly 70 years later (Chance & Frank, 1993), the first bioengineered commercial insulin analog during the 75th anniversary year for discovery of insulin. Awareness of immunological side effects of insulin therapy became apparent during the later forties. Later it was established that virtually all insulin treated diabetics have insulin antibodies (Berson et al., 1956; Berson & Yallow, 1959; Berson & Yallow, 1964). Severe insulin resistance developed in some patients due to the high level of antibodies (Berson & Yallow, 1960). These side effects were thought to be inevitable until the introduction of highly purified insulin in the early seventies.

Improvement in the insulin purity and quality of an insulin preparation was the first challenge and had been a goal since early
seventies. This advances have been made in increasing the purity. Introduction of Actrapid, the first neutral insulin solution, microcrystalline insulin—the first chromatographically purified insulin and recently, human insulin reflects success for the development of pure insulin preparations that does not induce production of antibodies (Skelbaek-Pedersen et al., 1987; Heding et al., 1980). The availability of human insulin represents an important advancement from the immunological point of view (Velcovsky & Federlin, 1984).

The important characteristics from the pharmaceutical point of view are the analytical control, as well as the chemical and physicochemical properties relevant to their formulation and efficacy (Stewart, 1974). Numerous attempts were made to retard the subcutaneous absorption of insulin to spare the diabetics from the chore and discomfort of multiple daily injections. Various approaches like cationic organic compounds, neutral suspensions, neutral suspension of insulin with zinc, alteration of the physical state and size of the suspended insulin-zinc particles and chemical derivation of insulin were adopted (Hallas & Moller, 1945; Schlichtkrull, 1958). Various protracted insulin preparations were explored like short acting, intermediate acting and long acting and biphasic formulations. Major milestones are the protamin insulin and protamine-zinc insulin (Scott & Fisher, 1936), isophane (NPH) insulin (Krayenbuhl & Rosenberg, 1946), lente insulins (Hallas et al., 1956) and biphasic insulin (Schlichtkrull et al., 1959). The crystallization of insulin and elucidation of the role of zinc for the association and structure of insulin have played an important role for this evolution (Abel et al., 1926; Hodgkin, 1974).

The chemical character of insulin was not known during the first years after the discovery of the hormone and it was natural to try different ways of administration, therefore a number of parenteral as well as several non-parenteral routes including cutaneous (Creque et al., 1980), nasal, lingual, tracheal (Wigley et al., 1971),
oral (Galloway & Root, 1972; Schlichtkrull et al., 1971), intestinal (Crane et al., 1986), rectal (Shichiri et al., 1978) and vaginal were tested. Insulin was administered most effectively by parenteral injection but due to the discomfort and inconvenience of injection therapy a great many efforts were made to improve the non-parenteral administration forms by protecting the insulin molecule from enzymatic degradation and facilitating the absorption by addition of various substances (Jensen, 1938). In recent years new formulation techniques have been used in an attempt to develop dosage forms of insulin applicable for absorption by oral (Galloway & Root, 1972; Schlichtkrull et al., 1971), nasal (Hirai et al., 1978), rectal (Shichiri et al., 1978) and other mucosal routes like buccal cavity (Ishida et al., 1981) with absorption promoting agents or in liposomes (Dapergolas & Gregoriadis, 1976; Tragl et al., 1979) have been tried repeatedly with very limited success.

Inspite of great successes mentioned above with respect to insulin preparations, the satisfaction was far behind from the clinical point of view. Newer problems emerged in due course. One of the problems being the development of insulin resistance irrespective of development of antibodies (Schlichtkrull et al., 1972; Reaven & Banting, 1988). Further on one hand, hyperinsulinaemia, the characteristics of insulin resistance and clinical fluctuation of insulin and glucose levels are the other problem. Insulin resistance can be due to various causes (Olefsky & Molina, 1990) and emerged as an important pathogenic factor in both type-1 and type-2 diabetes. Chronic lack of insulin, the primary pathogenic abnormality in type-I diabetic patients may lead to development of insulin resistance. Various studies have also reported that most insulin dependent (IDDM or type-I) diabetic patients may be moderately to severely resistant to the actions of insulin both at onset and after long duration of diabetes (Walker et al., 1963; Alford et al., 1971). In clinical practice, maintenance of insulin level is not the only target but it is also essential to maintain the glucose level,
other biochemical and hemodynamic parameters. It is not uncommon that, an over dose of insulin is generally results hypoglycemic effects. It is well known that death amongst diabetics is usually because of hypoglycemia rather than hyperglycemia (Edelman & Henry, 1995; Raue & Keim, 1999). Because of the insulin resistance and otherwise, the administration of oral hypoglycemics along with insulin is not uncommon.

Recently an alternation in trace elements in people with diabetes, have been reported, and this opened up new opportunities for improving nutritional management of diabetes (Freund et al., 1979; Brown et al., 1986; Mooradian & Morley, 1987). Various studies showed altered trace element metabolism in type 1 and type 2 diabetes mellitus in both human and experimental animal studies (Mooradian & Morley, 1987; Rossetti et al., 1990). Both deficiencies and excesses of metals have been observed (Mateo et al., 1978; Mather et al., 1979; Failla & Kiser, 1981; Johnson & Evans, 1984). Induced dietary deficiencies of individual trace metals in experimental animals have resulted in impaired insulin release, insulin resistance and glucose intolerance (Brown et al., 1975; Failla, 1983; Baly et al., 1984; Asayama et al., 1986). Conversely, supplementation of the deficient nutrient has been shown to improve glucose homeostasis in a number of studies (Rabinowitz et al., 1983b; Urberg & Zemmel, 1987). A few studies have also addressed the possibility of trace element supplementation as an adjunct therapy in human diabetic subjects, with mixed results (Niewoehner et al., 1986; Anderson et al., 1987; Clausen, 1988; Walter et al., 1991). In short, micro-nutrients are reported to possess beneficial effects in diabetes mellitus (Ernest et al., 1965; Anderson 1977, Nuttall 1983; Nuttall et al., 1984). Malnutrition has been suggested as a cause of diabetes mellitus in certain geographical areas (Golden & Golden 1983; Oli, 1983). However, the exact pathogenic role of malnutrition in diabetes mellitus has been disputed. Among various trace elements, vanadium and chromium...
have shown to have antidiabetic activity in various animal models and clinical studies. Vanadium, a trace element as a salt form or as a complex form was earlier reported to possess antidiabetic effects in various diabetic animal models (Shinde et al., 2002). Earlier our laboratory and various others have shown vanadium salts and few of its complexes to possess significant antidiabetic activity (Shinde et al., 2002). The antidiabetic activity of these agents found to ameliorate insulin resistance by improving insulin signaling and reduction in associated elevated lipid levels. Diabetes is associated with deranged hepatic and renal functions, vanadium salts and complexes earlier are reported to possess beneficial effects in diabetes by reducing the deranged renal and hepatic functions (Shinde et al., 2002).

Chromium, a group VIb transition element, in the form of chromium chloride and chromium picolinate are reported to possess antidiabetic activity by improving glucose intolerance, reducing fasting glucose, cholesterol and triglyceride levels (Shinde et al., 2003). Chromium supplementation is reported to produce improved impaired glucose and lipid metabolism (Urberg & Zemmel, 1987; Abraham et al., 1992). Chromium chloride and chromium picolinate is earlier reported to improve insulin sensitivity and hence reduce insulin resistance in various in-vivo and in-vitro models of diabetes (Shinde et al., 2003).

In the light of problems related to insulin resistance, hypoglycemia, fluctuations of glucose and insulin levels, the following objectives were set for present investigation.

1. To develop and optimize the insulin formulations with trace elements of chromium and vanadium.

2. To standardize the developed insulin formulations for various parameters like particle shape and size, redispersibility, pH, insulin content, metal ion content, insulin in supernatant solution and methyl paraben content.
3. Stability studies of the developed insulin formulations.

4. To evaluate the developed insulin formulations for antidiabetic activity with their effects on lipid profile, kidney function and liver function and various other biochemical parameters in STZ-induced type 1 diabetic rats.