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A COMPATIBILITY STUDY OF COMMON ADDITIVES IN PROTEIN HYDROLYSATE-DEXTROSE SOLUTIONS FOR PARENTERAL HYPERALIMENTATION

A Thesis

Presented to

The Faculty of the School of Pharmacy

University of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Norman Hideo Kobayashi

June 1973

This thesis, written and submitted by

Norman Hideo Kobayashi

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

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Chairman

Dated June 28, 1973

To Connie

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Finally, to my wife Connie, I dedicate this thesis.

N. H. K.

University of the Pacific
Stockton, California
June 1973

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I. INTRODUCTION

The use of the alimentary route to supply essential nutrients, either by mouth or nasogastric tube, is by far the best. This route, however, requires an intact and functioning gastrointestinal tract which may not be found in certain chronic intestinal diseases, malignant tumors, and cases of gastrointestinal obstruction. In these and certain other clinical situations, parenteral feeding is necessary to provide essential nutrients normally provided in the diet.

The intravenous route of administration is generally recognized as the most effective means of systemic administration. Yet, considering the other routes used clinically, it is of relatively recent development. Parenteral therapy began in 1616 with the discovery of the circulatory system by the English physician William Harvey (1). In 1656, Sir Christopher Wren successfully gave intravenous injections to dogs (2). Later years saw experimentation with attempts at parenteral therapy, especially blood transfusions, with little or no success. It was not until the early nineteenth century that Thomas Latta, in Scotland, used saline with great success to treat the intractable diarrhea of cholera. He is considered to be the first man to use intravenous injection in terms of rational therapy (2).

In the search for effective means of parenteral nutrition, various ingredients were infused into animals and man.

In 1873, Edward Hodder successfully injected milk into man (3). The intravenous injection of glucose for nutritional purposes was first accomplished by Kausch in 1911 (4). The most significant discovery for parenteral therapy was that of Francis Siebert who in 1923 discovered the cause of pyrogens in distilled water (5). In 1937, Rose's (6) identification of the amino acids essential for the growth of the rat was a significant step for parenteral nutrition. This discovery led to the development of the first protein hydrolysates for intravenous use in man. Meng and co-workers (7) studied parenteral nutrition in dogs. They injected carbohydrates, fat, protein, minerals, and vitamins for as long as ten weeks at a time. It was Dudrick (8) who made the major breakthrough for parenteral nutrition in 1967. He demonstrated, with the use of six beagle puppies, that the simultaneous administration of glucose and a nutritionally balanced mixture of amino acids from a protein hydrolysate, with added minerals and vitamins, would support an anabolic state. Muscular and skeletal growth were normal, as was body chemistry.

The terms intravenous or parenteral hyperalimentation are often used to describe this procedure because of the hyperosmolarity of the solution. More recently, total parenteral nutrition (TPN) has been used by various authors. Burke (9) has defined this method as the intravenous administration of proteins, calories, essential intracellular ions and other nutrients sufficient to achieve tissue synthesis and an anabolic state in patients where oral feeding is

impossible or, alone, inadequate. Flack and co-workers (10) have defined it in slightly different terms. They describe the process as the intravenous administration of sufficient nutrients above the usual basal requirements to achieve tissue synthesis, positive nitrogen balance, and anabolism.

A variety of protein hydrolysates which differ not only the starting protein but also their mode of preparation have been used. Geyer (21) has reported that, originally, investigators conducted experiments on hydrolysates of meat, fibrin, and milk. Casein or fibrin hydrolysates are extensively used at the present, but those prepared from lactalbumin, bovine serum protein and human serum albumin have reportedly been investigated as possible alternatives.

Ravin (13) has divided the clinical indications for parenteral hyperalimentation into five general categories:

1. Patients who cannot eat:
   - Esophageal carcinoma
   - Gastric carcinoma
   - Obstructive peptic ulcer
   - Paralytic ileus, etc.

2. Patients who should not eat:
   - Traumatic or inflammatory entercutaneous fistulas
   - Regional enteritis
   - Granulomatous colitis
   - Pancreatitis
   - Laryngeal incompetence, etc.

3. Patients who will not eat enough:
   - Postoperative geriatic patients
   - Anorexia nervosa, etc.

4. Patients who cannot eat enough:
   - Multiple fractures
   - Major full-thickness burns
   - Ulcerative colitis
   - Short bowel syndrome
   - Malabsorption syndrome, etc.

5. Infants who have congenital anomalies or chronic diarrhea.

In oral alimentation, essentially all dietary protein is hydrolysed to the free amino acids. The digestive enzymes of the gastrointestinal tract do contribute to his, but the major end products of their action are small peptides. These are resolved into free amino acids by peptidase present in the mucosal lining of the small intestine. Therefore, if the dietary source is restricted or insufficient in one or more of the essential amino acids, tissue protein synthesis for growth and healing will be restricted and some of the amino acids will enter catabolic pathways leading to formation and excretion of nitrogen, primarily in the form of urea (9). In healthy individuals, a few days of reduced nutritional intake is of little consequence, but when a patient is subject to major trauma or disease, catabolism occurs at a much faster rate (16). For example, if a patient is under physiological stress due to infection or trauma, the caloric requirement increases to 3,000 to 4,000 calories per day (22).

NUTRITIONAL REQUIREMENTS

Proteins and Amino Acids

There are many nitrogen sources that have been used (15); amino acids, protein hydrolysates, albumin, plasma, and whole blood. Albumin, plasma, and whole blood have been quite useful in restoring and maintaining intravascular oncontic pressure, but the protein provided in this manner must undergo time and energy consuming conversions to amino acids for protein

synthesis. Until fairly recently, crystalline amino acids have been too costly when adequately refined for intravenous use. Therefore, protein hydrolysates from enzymatic hydrolysis of casein or fibrin have been the major sources of intravenous nitrogen (see Table I). These solutions provide about 60% of the available nitrogen as amino acids and 40% as dipeptides and tripeptides (15). The importance of maximum amino acid content and minimum polypeptide content has been established. However, no controlled clinical studies, comparing the efficacy of protein hydrolysates and synthetic amino acids, can be found in the literature. Asch, et al. (38), in five infants and two small children receiving alternate courses of therapy with casein hydrolysate and the synthetic amino acid solution, found little difference with regard to nitrogen balance and weight gain. However, the small number of patients prevents any meaningful statistical inference.

Twenty-two amino acids (11) are ordinarily required for synthesis of tissue proteins, and the absence of any one of them could prevent the formation of a polypeptide. With no intake of any essential amino acid, tissue synthesis will not occur. The synthesis increases in proportion to the level of the amino acids which is fed, up to an optimal point (12).

The 7% Protein Hydrolysate Injection U.S.P.[a] used in this study is an aqueous solution of a pancreatic (porcine) enzymatic digest of casein. It has been fortified by the

---

a - C.P.H., Cutter Laboratories, Berkeley, Ca.

TABLE I

Solutions Available Commercially

For Parenteral Hyperalimentation

Basic Solution	Total Nitrogen Gm/L	Total Protein Equivalent Gm/L
8.5% Crystalline Amino Acids[a] 500 ml plus 50% Dextrose to make 1000 ml	6.25	39.1
5% Fibrin Hydrolysate[b] 750 ml plus 50% Dextrose to make 1000 ml	5.06	31.6
7% Casein Hydrolysate[c] 590 ml plus 50% Dextrose to make 1000 ml	6.10	38.1
10% Casein Hydrolysate[d] 500 ml plus 50% Dextrose to make 1000 ml	6.80	42.5

a - FreAmine, McGaw Laboratories, Glendale, Ca.

b - Aminosol, Abbott Laboratories, North Chicago, Ill.

c - C.P.H., Cutter Laboratories, Berkeley, Ca.

d - Hyprotigen, McGaw Laboratories, Glendale, Ca.

addition of quantities of methionine, tryptophan, and phenylalanine. It is interesting that Wunner and co-workers (23) have reported that tryptophan causes a non-specific increase in the rate of protein synthesis and net deposition of protein associated with an increase in polyribosomes and a decrease in monoribosomes in the liver. Conversely, feeding a mixture deficient in other amino acids, did not decrease liver protein synthesis in the normal animal. Each 100 ml of C.P.H. contains 4.89 Gm protein hydrolysate which contains all the essential amino acids in the following approximate amounts, expressed in Gm/L:

Leucine	4.15	Arginine	1.5
Valine	3.0	Threonine	1.8
Lysine	3.5	Methionine	2.2
Isoleucine	2.4	Histadine	1.2
Phenylalanine	2.3	Tryptophan	0.5

In addition, the following non-essential amino acids are present as a result of the enzymatic digestion of casein: glutamic acid, proline, serine, aspartic acid, cystine, alanine, tyrosine, and aminoacetic acid. The pH has been adjusted with lactic acid to about 5.3. Electrolytes are found in the formulation in the following respective mEq/L ion concentrations: sodium 23, potassium 11, calcium 4, chloride 8, and phosphate 8.

Estimates have been made on the protein and amino acid requirements of man (16,17). Based on these nitrogen balance studies, an intake of 0.45 Gm protein per Kg body weight would be needed by the average adult to cover urinary, fecal,

cutaneous, and other minor routes of nitrogen excretion (18). DeMaeyer and Vanderbrought (19) have calculated that a child of 3 to 5 years needs an average of 0.6 to 0.7 Gm of protein per Kg body weight. There are few relevant studies on the protein requirements among the elderly. Kountz and co-workers (20) suggest that their requirements for nitrogen equilibrium averages 0.6 Gm per Kg body weight.

Carbohydrates

In addition to the protein/amino acid requirements, an additional source of calories is required. Preaston (14) has stated that 25 to 30 cal/Kg are required daily for maintenance purposes alone. In a 70 Kg man, the body usually stores approximately 100 to 200 Gm of carbohydrates chiefly in the form of glycogen which may be utilized in 24 hours or less (12). After exhaustion of these stores, breakdown of body tissue occurs unless adequate calories are supplied. This results in muscle wasting, delayed wound healing, lowered resistance to infection, and prolonged convalescence(14). It has been recommended that an extra 13% of caloric requirement be added for the hyperthermic patient for each degree centigrade above normal body temperature (14). Considering the caloric content of each of the three energy sources, fat is by far the best, yielding 9 cal/Gm of fat which can be administered as an emulsion (soya bean oil emulsified by egg phosphatide made isotonic with glycerin). Carbohydrates and protein each yield 4 cal/Gm (14). It appears to make little difference whether the calories are administered as fats, carbohydrates,

or even alcohol, provided that at least 150 calories are supplied per Gm of nitrogen (protein or amino acid) infused (15). Hypertonic dextrose offers the most inexpensive and practical caloric source at the present time.

Minerals

The mineral requirement can be divided into two major classifications: major electrolytes and trace elements. The major electrolytes would include sodium, chloride, bicarbonate, potassium, calcium, magnesium, phosphate, and possibly sulfate. Trace elements include: iron, zinc, copper, iodide, fluoride, chromium, cobalt, manganese, molybdium, selenium, and vanadium (24).

Sodium, potassium, and chloride ions are probably the most critical of the electrolyte additives. It has been reported (24) that their concentrations should be monitored daily and sometimes more frequently in cases of serious electrolyte and fluid imbalances. Therefore, no single value or range can be followed, as the requirements will vary depending on renal, cardiovascular, and endocrine condition of the patient. The following additive levels have been reported as average adults doses in hyperalimentation: sodium (as chloride) 40-50 mEq/L, potassium (as chloride) 30-40 mEq/L, and chloride 50 mEq/L (10,26).

Potassium plays an essential role in the transportation of glucose and amino acids across the cell membrane (27). Also, if this ion is not administered, potassium from the bloodstream will be used to accompany glucose and amino acids into the cell,

creating a serum deficiency. Grant, et al. (27) report that as much as 160 to 200 mEq of potassium may be given with 4,000 calories for effective utilization of the carbohydrate. Therefore, increased amounts of glucose and amino acids administration would seem to require increased amount of potassium in order to be absorbed by the cell and to maintain the proper electrolyte balance.

There appears to be no hormonal mechanism for controlling serum magnesium levels (24). Normally, serum levels must be dependent primarily on intake and excretion through the kidney. It is the second most plentiful cation within cellular fluids (28). Guyton (29) states that magnesium is required as a catalyst for many intracellular enzymatic reactions, particularly those relating to carbohydrate metabolism. The recommended daily allowance for an adult on total parenteral nutrition has been reported as 25 mEq/day (24) or, on the liter basis, 8 mEq/L (26). It is usually admixed into the solution as the sulfate salt.

Calcium ion has been commonly added as the gluconate or glucoheptonate salt. Calcium is reported to be normally present in small quantities in the extracellular fluid and to a minor extent in the structure and cytoplasm of cells of soft tissue (28). Calcium is also essential for muscle contraction, nerve condition, and blood coagulation. Davidson (30) states that the bulk of the body's calcium and phosphorus is located in the bones. This accounts for 99% of the total calcium and 80 to 85% of the total phosphorus, which is in combination

with calcium. The remaining phosphorus (i.e., phosphate) exists as a major anion of intracellular fluid. The phosphate ion is generally admixed in the form of potassium acid phosphate.

Casein hydrolysates are reported to have 100 to 125 mg/L of calcium, approximately six times that of fibrin hydrolysates (24). There are approximately 10 mg/L of phosphorus as inorganic phosphate in the fibrin, as compared to 200 mg of phosphorus as free inorganic phosphate in the casein hydrolysate.

There have been two schools of thought concerning the addition of calcium and phosphate to hyperalimentation solutions. Some authorities believe that they should be added routinely and others believe the serum levels should be followed and the ions added when indicated. The actual significance of calcium administration has been questioned because of the development of disuse osteoporosis in patients who are bedridden for long periods of time (31,32). These conditions result in increased urinary and fecal calcium levels. Some authorities also feel that administration of calcium is necessary when phosphate is given in order to prevent hypocalcemia. Allen and co-workers (33) have reported hypocalcemia in some hypophosphatemic patients given phosphorus in doses of 4 to 8 mEq over eight hours. Inorganic phosphate is required for the production of high energy organic phosphates. Lotz (34) and Lichtman (35) have reported symptoms in hypophosphatemia such as malaise, muscle weakness, and mental depression.

Kaminiski (26) has expressed the approximate adult requirement for calcium to be 10 mEq/L and phosphate to be 20 mEq/L.

Sulfur present in methionine, cysteine, and taurine (23) is apparently sufficient to form other sulfur containing compounds provided the supply of these amino acids is sufficient.

To supply the trace elements, some authorities (25) feel the periodic infusion of plasma or blood is sufficient for normal body needs. Dudrick, et al. (36) and Doolas (37) have stated that trace elements are needed only after one month of treatment with total parenteral nutrition.

Vitamins

Guyton (29) has defined a vitamin as an organic compound needed in small quantities for operation of normal bodily metabolism and that cannot be manufactured in the cells of the body. Greene (39) stated that since they are intimately involved in metabolism of carbohydrates, proteins, and fat, they are an essential additive to complete parenteral nutrition.

The use of an injectable multiple vitamin[a] consisting of ascorbic acid, vitamin A, vitamin D, thiamine HCl, riboflavin, pyridoxine HCl, niacinamide, dexpanthenol, and vitamin E has resulted in vitamin A and D toxicity (40,41). To avoid this, it has been recommended that a change be made to a combination of B complex with ascorbic acid[b] after about five days of total parenteral nutrition (42).

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a - M.V.I., U.S.V. Pharmaceutical Corp., Tuckahoe, N. Y.

b - Solu-B Forte, The Upjohn Company, Kalamazoo, Mich.

Optional additives, which depend upon the clinical indications are phytonadione, cyanocobalamin, and folic acid (10,13). McGovern (22) has reported the addition of corticosteroids in some cases of inflammatory enteritis.

Geyer (44) has stated that low doses of insulin were given to aid sugar uptake by the tissues, thereby decreasing urinary loss. Doolas stated (37) that exogenous insulin is not usually required, since endogenous insulin production is stimulated by dextrose and most amino acids. He further states that in the early postoperative period, insulin may be needed, since postoperative patients develop a transient elevation in blood glucose. Diabetic patients, of course, will require exogenous insulin. DeSchepper (45) has reported that insulin enhances intracellular utilization of amino acids in protein synthesis, rather than increase the net uptake of amino acids. It has been reported (46) that the addition of insulin to intravenous systems has resulted in 23-28% adsorption of the drug to glass and plastic (46). The reported levels of regular insulin used range from 20 to 40 units per liter (42,27).

COMPATIBILITY STUDIES

There are very few scientific studies on the compatibility of hyperalimentation solutions reported in the literature. Because of this, there is little or no data on which to base decisions of compatibility and/or stability of various admixtures. Those studies that are available are either the result of a personal experience, without controls, or conjecture on what would be expected.

Cluxton (44) has elaborated on the complexities of making compatibility studies on hyperalimentation solutions. He cites the variability of each formula as well as variation in concentration of each ingredient as a problem in experimental design. He points out that it is dangerous to assume that because there is no evidence of physical incompatibility, that a solution is compatible.

Patel and Phillips (45) compiled a compatibility chart based on published articles and actual experimentation. Their study consisted of mixing different solutions on a glass slide and examining the slide under a microscope. Evaluation of physical compatibility was based on the clarity of the resulting solution. This study pointed out that order of mixing can affect the compatibility. Chlortetracycline, nitrofurantoin, novobiocin, pentobarbital sodium, and thiopental sodium were found to be incompatible with protein hydrolysates.

Pelissier and Burgee (46), in a compilation of information from published articles, manufacturers' intravenous compatibility charts, manufacturer's package inserts, and brochures, report that albumin (human), aminophyllin, sodium salts of barbiturates, Cedilanid-D, chlortetracycline, corticotropin, digitoxin, digoxin, diphenhydramine, EDTA, epinephrine, ergonovine, erythromycin, fibrinolysin, hydralazine, metaraminol, nitrofurantoin, novobiocin, and protein (human) as incompatible. In a preliminary study (47), it found that corticotropin, hydrocortisone sodium phosphate, and hydrocortisone sodium succinate were physically compatible with protein hydrolysate,

but methylprednisolone sodium succinate was physically incompatible.

Williams and Moravec (48,49) and Fowler (50) have reported several drugs as incompatible with protein hydrolysates, based on the recommendation from the manufacturer that no drugs be added to the solutions or that only certain diluents be used. These studies list only these drugs as compatible with protein hydrolysates: alcohol, ascorbic acid, vitamin B complex, and thiamine. Among the drugs list as incompatible, it is interesting that these have been listed as incompatible: corticotropin, calcium gluconate, cortisone acetate, heparin sodium, insulin (regular), magnesium sulfate, hydrocortisone sodium succinate, cyanocobalamin, and phytonadione.

Cohon (51) mentions that manufacturers recommend that other drugs not be added to protein hydrolysates. He cautions that protein binding may occur, particularly with antibiotics.

A most comprehensive examination of electrolyte compatibility with 8.5% synthetic amino acid solution[a] has been performed by Collin, et al. (52). Initially, they studied the following electrolytes in these concentrations: magnesium sulfate, 10-40 mEq/L, potassium phosphate, 20-100 mEq/L, calcium gluceptate, 10-50 mEq/L, and sodium bicarbonate, 20-100 mEq/L. The reproducible precipitation observed, was identified analytically and spectroscopically as a calcium-phosphate

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a - FreAmine, McGaw Laboratories, Glendale, Ca.

complex. It was found that calcium (as gluceptate) and phosphate (as potassium acid) are compatible to some extent. They found this compatibility problem can be avoided if calcium and phosphate ions are added to the 50% dextrose and 8.5% amino acid solution, respectively, prior to their mixing. The precipitation can also be avoided by insuring that calcium ion is the last electrolyte added to the final dextrose-amino acid solution. At least 30 mEq/L of potassium phosphate were found to be compatible with 15mEq/L of calcium gluceptate with these two procedures.

Burke (58) has elaborated on ten commonly used additives, based solely on his own personal experience. Albumin, which is normally listed as incompatible, was found to be stable and clinically effective for at least 24 hours. Calcium is reported to be incompatible with sodium bicarbonate, as is magnesium sulfate. Burke also reports that cyanocobalamin, and folic acid are inactivated by ascorbic acid, with cyanocobalamin also being inactivated by high concentrations of dextrose. He does note that one should conduct his own controlled tests prior to admixing any drug in a hyperalimentation solution.

As parenteral nutrition becomes more common in medical practice, it becomes imperative that more information be made available on possible admixture incompatibilities. Thus, the object of this study was to determine the compatibility of the majority of common additives with a mixture of 590 ml 7% Protein Hydrolysate Injection U.S.P. and 410 ml 50% Dextrose

Injection U.S.P. A physical evaluation alone (i.e., observation for precipitation, color change, or evolution of gas) with pH monitoring was used in the preliminary study. Ultra-violet spectroscopy and thin-layer chromatography were used to aid in detecting alterations in the chemical structure of the protein hydrolysate. These alterations may be indicative of unseen chemical incompatibility.

II. EXPERIMENTAL METHOD

The purpose of this project was to study the admixture compatibility of common additives with a mixture comprised of 590 ml 7% Protein Hydrolysate Injection U.S.P. and 410 ml 50% Dextrose Injection U.S.P. (Polynute™ Pack)[a]. The experimental work was divided into two sections: physical compatibility and chemical compatibility.

PHYSICAL COMPATIBILITY

The object of the physical compatibility study was to determine the maximum concentration of electrolytes which may be admixed with Polynute without physical evidence of incompatibility (precipitation, evolution of gas, or color change). A maximum compatible concentration was evaluated with other additives, such as selected vitamins, insulin, and heparin.

Each liter of Polynute was prepared by infusing 410 ml of the 50% Dextrose Injection into 590 ml of the 7% Protein Hydrolysate, utilizing the transfer set provided in the Polynute pack. This mixed solution was incorporated into the physical study within eight hours as recommended by the manufacturer's literature.

Each additive-hydrolysate combination was prepared in duplicate, utilizing 25 ml quantities in 50 ml erlenmeyer

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a - Cutter Laboratories, Inc., Berkeley, Ca.

flasks for each concentration-combination. The transfer of additives was made with graduated pipettes. The resulting solutions were stored at room temperature (22° C) under normal fluorescent room lighting. Physical evaluation was based on visual examination and pH readings taken at intervals of one, four, eight, and twenty-four hours after admixture. The pH readings were made using a Corning pH meter, Model 7[a].

Calcium gluconate[b], magnesium sulfate[c], and potassium phosphate[d], were the electrolyte additives selected for this study. These electrolytes were first evaluated individually with Polynute. Following this, combinations of the electrolytes were added to the Polynute solution, starting at a concentration of 4 mEq/L, then in increments of 10 mEq/L to a level of 100 mEq/L or until physical evidence of incompatibility was observed.

The following vitamins were evaluated for physical compatibility: vitamin B complex with C (Solu B Forte[e]), folic acid (Folvite[f]), phytonadione (AquaMephyton[g]),

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a - Corning Scientific Instruments, Corning, N. Y.

b - Pasadena Research Laboratories, Pasadena, Ca.

c - Eli Lilly and Company, Indianapolis, Ind.

d - McGaw Laboratories, Glendale, Ca.
The potassium phosphate used in this study contained potassium dihydrogen phosphate 0.9 Gm and potassium monohydrogen phosphate 4.7 Gm in each 30 ml. All future references to potassium phosphate will indicate this information.

e - The Upjohn Company, Kalamazoo, Mich.

f - Lederle Laboratories, Pearl River, N. Y.

g - Merck Sharp and Dohme, West Point, N. Y.

cyanocobalamin (Rubramin PC[a]), and multiple vitamin infusion (M.V.I.[b]). Each vitamin was evaluated individually with each of the electrolytes and in combination with the maximum concentration of electrolytes found to be compatible.

Regular insulin (Iletin[c]) and sodium heparin[d] were also evaluated in combination with maximum compatible concentrations of electrolytes and vitamins.

Cluxton (44) has calculated the sample size required for each sample for a two-tailed significance level of 5 percent to be approximately three. This is assuming that 10% of the admixutres we prepare are incompatible. If we assume that 5% of the admixtures are incompatible, this results in a sample size of less than two. During this physical evaluation, a sample size of two (i.e., duplicate) was used for each combination; however, during the chemical analysis, n=3 was used. Cluxton further suggests that the two following assumptions be made: 1) if a high concentration of an ingredient is compatible, a lesser concentration will also be stable and 2) order of addition is not important. The later assumption is, of course, not valid, especially in light of the report of Collin, et al., but essential because of the magnitude of the combinations that develop with the variation of the order of mixing. A random order was selected and maintained

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a - E. R. Squibb and Sons, New York, N. Y.

b - U.S.V. Pharmaceutical Corp., Tuckahoe, N. Y.

c - Eli Lilly and Company, Indianapolis, Ind.

d - Invenex Laboratories, San Francisco, Ca.

during the study.

CHEMICAL COMPATIBILITY

Since pH change, clarity of solution, precipitation, or color change often fails to detect alteration or inactivation of ingredients, ultra-violet spectroscopy (U.V.) and thin-layer chromatography (TLC) were employed to elucidate possible chemical changes that may have taken place.

U.V. Spectroscopy

U.V. absorption spectra were obtained utilizing the Bauch and Lomb Spectronic 600[a] double beam spectrophotometer. A Liner/Log Varicord 43[b] recorder provided recordings of the spectra. Samples were measured in Spectrosil[c], 10 mm cells using a dueterium lamp to provide the incident beam wavelength range of 220 to 320 nanometers.

The same vitamins, calcium gluconate, and potassium phosphate were examined spectrophotometrically. Magnesium sulfate did not exhibit any compatibility problems with other electrolytes and, therefore, was not considered. Because of the number of additives present when insulin and heparin were admixed, it was felt any spectrophotometric result would be inconclusive. Therefore, these combinations were not examined spectrophotometrically.

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a - Bauch & Lomb Optical Company, Rochester, N. Y.

b - Photovot Corporation, New York, N. Y.

c - Perkin-Elmer, Coleman Instrument Division, Maywood, Ill.

Aqueous dilutions of the Polynute were made to determine the concentration required to obtain absorbance values of 0.3 to 0.9. This dilution was used as the standard by which all subsequent spectra were compared. All admixture combinations were examined at this dilution concentration regardless whether the additive was spectrophotometrically decipherable at that concentration.

It was decided to examine each vitamin individually against each selected electrolyte solely at therapeutic concentrations and in combination (both calcium and phosphate) at therapeutic concentration and a maximum compatible concentration. Each reference contained water, electrolyte and the vitamin while each test sample contained Polynute, electrolyte(s), and vitamin. This allows scanning for the Polynute only. The references were prepared singly and the samples in triplicate. The respective samples were examined at one, four, eight, and twenty-four hours. This was facilitated by removal of 2.5 ml from each sample and its corresponding reference and diluting with distilled water to 50 ml. The results of the spectrophotometric measurements of these admixtures were compared with the standard spectra to detect any alteration in the protein hydrolysate. If there were any alterations in spectra, additional combinations were examined to determine further which additive may have been responsible for the spectral change.

THIN-LAYER CHROMATOGRAPHY

Basic methods used in this TLC procedure were developed

by Frye (53) in his investigation of degradation products of the amino acids in foodstuffs derived from soybean meal. Changes in the procedure were made to accommodate the Polynute.

Plates. Pre-coated glass TLC plates of cellulose (without fluorescence indicator)[a] were used. The 20 x 20 cm plates had a layer thickness of 0.10 mm.

Spotting Equipment. Drummond microcapillaries[b] of 25 microliter capacity were used. Application of approximately 0.5 microliter on the plate was facilitated by touching the capillary tip to surface of the plate in such a manner that the spot size was never larger than 2.0 mm. A DeSaga spotting template was used.

Developing Tanks. DeSaga-Heidelberg tanks with ground glass tops were used. To insure saturation, two sheets of 20.5 cm filter paper[c] were used to line two sides of the tank. A maximum of two TLC plates could be developed in each solvent system at one time.

Visualization Equipment. An Ensco-Calab[d] spraying apparatus which utilized a positive pressure source was used to coat the plates with the developing solution. A distance

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a - E. Merck and Co., Darmstadt, Germany; marketed by EM Laboratories, Elmsford, N. Y.

b - Kensington Scientific Corp., Emeryville, Ca.

c - Braun-Knecht-Hermann Co., San Francisco, Ca.

d - Metheson Scientific, Hayward, Ca.

of 20 to 25 cm was maintained between the plate and sprayer.

Drying Oven. A Thelco Model 27[a] was used to dry the TLC plates after developing in solvent system I and solvent system II. A setting of 3.5 maintained the oven at constant temperature of 100° C. Reducing the setting to 3.0 resulted in a temperature of 80° C. This setting was used for the drying of the plates after spraying.

Chemicals and Reagents. The solvents and chemicals used were of reagent grade or better. They were used directly without further attempts at purification.

Stepwise Procedure

1. Spot approximately 0.5 microliter on the TLC plate approximately 1.5 cm from the bottom, 2 cm from the right edge. Since the spot size should be kept below 2 mm in diameter, a hair dryer was used to dry the spot and prevent its spread.
2. The plate is carefully placed in the first solvent system. The tank has previously been filled and allowed to equilibrate. Approximately 80 to 100 ml of solvent system I is sufficient to fill the developing tank. Develop for 8 hours.

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a - Precision Scientific, Chicago, Ill.

Solvent System I:

2-propanol	60 parts
Butanone	15 parts
1 N Hydrochloric acid	25 parts

3. Remove the plate from the solvent system I and dry in the oven at least 5 minutes at 100° C to insure removal of all traces of hydrochloric acid.

4. Allow the plate to cool, then repeat step 2 in solvent system II. Develop for 6 hours.

Solvent System II:

2-methyl-2-butanol	50 parts
Butanone	20 parts
Propanone	10 parts
Methanol	5 parts
Water	15 parts
Aqueous ammonia (27%)	5 parts

5. Again dry the plate at 100° C for five minutes and allow to cool.

6. Spray the plate in the hood with freshly prepared cadmium acetate-ninhydrin spray reagent.

Prepare cadmium acetate solvent by adding 0.5 Gm of reagent grade cadmium acetate to a mixture of 50 ml of water and 10 ml glacial acetic acid. Add enough propanone to prepare a final volume of 500 ml. For use, enough solid ninhydrin was added to proportions of the above solution to make the final concentration of ninhydrin equal to 0.2% w/v. It was found that 100 ml was a convenient quantity to prepare when

developing a small number of plates.

7. Dry the sprayed plate for fifteen minutes at 80° C to effect visualization. The plate should be dried on their sides, not flat.
8. Document the chromatogram by recording the spotting pattern with tracing paper.

III. RESULTS

PHYSICAL COMPATIBILITY

The physical compatibility evaluation of selected electrolytes and vitamins in Polynute was based on visual examination and pH readings. These were taken at intervals of one, four, eight, and twenty-four hours following admixture. All determinations were performed in duplicate. It was observed that there was little variation in pH between intervals, therefore, all pH readings reported are averages of the eight readings, unless otherwise stated. Without additives, Polynute had a pH of 5.22. If, during any of these time examinations, physical compatibility was observed, the combination was considered incompatible.

ELECTROLYTES

The electrolytes represented on the left margin of Tables II to XI were admixed first with the Polynute. Quantities of electrolytes shown in these tables are the amounts added to the Polynute and do not include those amounts already present. In these tables, the symbol "C" is used to denote the absence of any visual change in the solution and, therefore, denotes physical compatibility. The symbol "X" signifies the formation of a visible precipitate, thus physically incompatible.

There appears to be no physical incompatibility with

the following combinations of electrolytes: calcium gluconate-magnesium sulfate (Table II) and potassium phosphate-magnesium sulfate (Table IV). A maximum compatible concentration was reached with the calcium gluconate-potassium phosphate combination (Table VI). Reversing the order of mixing did not appear to affect the physical compatibility except in the case of calcium gluconate and potassium phosphate (Table VIII). The addition of magnesium sulfate to the calcium-phosphate combinations did not appear to alter the appearance of the solutions (Table X). As the concentration of the additive increase, there was a corresponding change in the pH of the Polynute, reflecting the pH of the additives.

Magnesium Sulfate (mEq/L)

Calcium Gluconate (mEq/L)	0	4	6	8	10	20	30	40	50	100
0										
4		C								
6			C							
8				C						
10					C					
20						C				
30							C			
40								C		
50									C	
100										C

Table II. Physical Compatibility of Calcium Gluconate and Magnesium Sulfate, Admixed in Polynute.

Magnesium Sulfate (mEq/L)

Calcium Gluconate (mEq/L)	0	4	6	8	10	20	30	40	50	100
0	5.22									
4		5.22								
6			5.22							
8				5.22						
10					5.22					
20						5.22				
30							5.22			
40								5.22		
50									5.24	
100										5.26

Table III. pH of Solutions Containing Calcium Gluconate and Magnesium Sulfate, Admixed in Polynute.

Magnesium Sulfate (mEq/L)

Potassium Phosphate (mEq/L)	0	4	6	8	10	20	30	40	50	100
0										
4		C								
6			C							
8				C						
10					C					
20						C *				
30							C *			
40								C		
50									C	
100										C

Table IV. Physical Compatibility of Potassium Phosphate and Magnesium Sulfate, Admixed in Polynute.

*Readings taken at one and four hours only.

Magnesium Sulfate (mEq/L)

Potassium Phosphate (mEq/L)	0	4	6	8	10	20	30	40	50	100
0	5.22									
4		5.35								
6			5.46							
8				5.47						
10					5.55					
20						6.03*				
30							6.05*			
40								6.13		
50									6.17	
100										6.35

Table V. pH of Solutions Containing Potassium Phosphate and Magnesium Sulfate, Admixed in Polynute.

*Average of readings taken at one and four hours only.

Calcium Gluconate (mEq/L) \ Potassium Phosphate (mEq/L)	0	4	6	8	10	15	20	25	30	35	40	50
0												
4		C										
6			C									
8				C	C*							
10					C							
15					C*	C	C*	C*				
20					C*	C	C	X	X	X	X	
25					C*	C	X*	X	X*	X	X*	
30						X	X	X*	X	X*	X*	
35						X	X*	X	X*	X	X*	
40						X	X*	X*	X*	X*	X	
50							X		X		X	X

Table VI. Physical Compatibility of Calcium Gluconate and Potassium Phosphate, Admixed in Polynute.

*Readings taken at one and four hours only.

Calcium Gluconate (mEq/L) \ Potassium Phosphate (mEq/L)	0	4	6	8	10	15	20	25	30	35	40	50
0	5.22											
4		5.35										
6			5.49									
8				5.55								
10					5.84							
15						5.85						
20						5.60	5.86	5.85	6.04	6.08	6.15	
25						5.63		5.96		6.08		
30						5.62	5.84	6.14	6.02			
35						5.58		5.95				
40						5.70	5.76				6.14	
50							5.77		5.96		6.05	6.18

Table VII. pH of Solutions Containing Calcium Gluconate and Potassium Phosphate, Admixed in Polynute.

Potassium Phosphate (mEq/L) \ Calcium Gluconate (mEq/L)	0	25	35	40	50
0	C				
25		C	C		
35		C	C		
40				C	
50					X[a]

Table VIII. Physical Compatibility of Potassium Phosphate and Calcium Gluconate, Admixed in Polynute. (Note order of mixing reverse of Table VI.)

a - Precipitate not observed until 24th hour.

Potassium Phosphate (mEq/L) \ Calcium Gluconate (mEq/L)	0	25	35	40	50
0	5.22				
25		5.90	6.00		
35		5.90	6.00		
40				6.18	
50					6.22

Table IX. pH of Solutions Containing Potassium Phosphate and Calcium Gluconate, Admixed in Polynute. (Note order of mixing reverse of Table VII.)

Potassium Phosphate (mEq/L)

Calcium Gluconate (mEq/L) and Magnesium Sulfate (mEq/L) each	0	4	6	8	10	20	30	40	50
0									
4		C							
6			C						
8				C					
10					C				
20						C			
30							X		
40								X	
50									X

Table X. Physical Compatibility of Calcium Gluconate and Magnesium Sulfate with the addition of Potassium Phosphate, Admixed in Polynute.

Potassium Phosphate (mEq/L)

Calcium Gluconate (mEq/L) and Magnesium Sulfate (mEq/L) each	0	4	6	8	10	20	30	40	50
0	5.22								
4		5.24							
6			5.34						
8				5.40					
10					5.49				
20						5.78			
30							5.94		
40								6.04	
50									6.08

Table XI. pH of Solutions Containing Calcium Gluconate and Magnesium Sulfate with addition of Potassium Phosphate, Admixed in Polynute.

VITAMINS

The vitamins were examined at two concentrations. An order of mixing was chosen at random and maintained throughout the procedure. The calcium gluconate was added first, then potassium phosphate, magnesium sulfate, and the vitamin. As with the electrolytes, as the concentration of the vitamin was increased, the pH of the solution reflected the pH of the additive. The addition of the vitamin did not alter the concentration at which each electrolyte was found to be compatible (Table XII).

INSULIN AND HEPARIN

Regular insulin was added to the Polynute in increments of 20 units to a level of 100 units/L. Insulin was admixed last with the following additives already present in the Polynute: calcium, magnesium, phosphate, phytonadione, cyanocobalamin, and folic acid. One set was prepared with M.V.I. and one with Solu B Forte, assuming that both vitamin supplements would not be administered simultaneously. These admixtures were prepared by adding the components to the Polynute in the following sequence: calcium gluconate, potassium phosphate, magnesium sulfate, M.V.I. or Solu B Forte, folic acid, cyanocobalamin, phytonadione, and finally the insulin.

Heparin sodium was added to the Polynute in similar sequence and used with the insulin, adding the heparin as the last additive. A concentration of 1000 mcg/L was used. The addition of regular insulin or heparin sodium did not alter the appearance of the solution (Table XIV, XV). There was also little change in the pH of the solution.

	Ca 5 mEq/L	Ca 100 mEq/L	Mg 5 mEq/L	Mg 100 mEq/L	Phos. 10 mEq/L	Phos. 100 mEq/L		Ca & Phos. 20 mEq/L	Ca, Mg, & Phos. 20 mEq/L	Ca & Phos. 30 mEq/L
Solu B Forte 5 ml/L	C	C	C	C	C	C		C	C	X
10 ml/L	C	C	C	C	C	C		C	C	X
Folic Acid 0.5 mg/L	C	C	C	C	C	C		C	C	X
5 mg/L	C	C	C	C	C	C		C	C	X
Cyanocobalamin 300 mcg/L	C	C	C	C	C	C		C	C	X
1000 mcg/L	C	C	C	C	C	C		C	C	X
Phytonadione 10 mg/L	C	C	C	C	C	C		C	C	X
50 mg/L	C	C	C	C	C	C		C	C	X
M.V.I. 10 ml/L	C	C	C	C	C	C		C	C	X
20 ml/L	C	C	C	C	C	C		C	C	X

Table XII. Physical Compatibility of Selected Vitamins in the Presence of Calcium, Magnesium, and/or Phosphate, Admixed in Polynute.

	Ca 5 mEq/L	Ca 100 mEq/L	Mg 5 mEq/L	Mg 100 mEq/L	Phos. 10 mEq/L	Phos. 100 mEq/L		Ca & Phos. 20 mEq/L	Ca, Mg, & Phos. 20 mEq/L	Ca & Phos. 30 mEq/L
Solu B Forte 5 ml/L	4.98	5.08	4.98	4.98	5.30	6.50				5.85
10 ml/L	4.85	4.95	4.83	4.77	5.10	6.48		5.45	5.35	5.65
Folic Acid 0.5 mg/L	5.22	5.25	5.20	5.05	5.55	6.50				6.00
5 mg/L	5.25	5.23	5.20	5.05	5.58	6.50		5.85	5.70	6.00
Cyanocobalamin 300 mcg/L	5.22	5.30	5.23	5.10	5.63	6.52				6.05
1000 mcg/L	5.22	5.30	5.23	5.10	5.63	6.52		5.85	5.72	6.05
Phytonadione 10 mg/L	5.30	5.35	5.25	5.15	5.65	6.60		5.85	5.75	6.15
50 mg/L	5.25	5.35	5.25	5.15	5.65	6.60				6.15
M.V.I. 10 ml/L	5.28	5.35	5.23	5.22	5.58	6.55		5.82	5.72	6.10
20 ml/L	5.25	5.32	5.20	5.10	5.52	6.53				6.10

Table XIII. pH of Solutions Containing Selected Vitamins in the Presence of Calcium, Magnesium, and/or Phosphate, Admixed in Polynute.

Polynute with: Folic Acid 5 mg/L, B-12 1000 mcg/L, K-1 10 mg/L, plus:	Ca 5 mEq/L	Ca 100 mEq/L	Mg 5 mEq/L	Mg 100 mEq/L	Phos. 10 mEq/L	Phos. 100 mEq/L		Ca & Phos. 20 mEq/L each	Ca, Mg, & Phos. 20 mEq/L each
M.V.I. 10 ml/L	C	C	C	C	C	C		C	C
pH	5.22	5.22	5.10	5.62	6.50	5.67		5.67	5.80
Solu B Forte 10 ml/L	C	C	C	C	C	C		C	C
pH	5.00	5.05	4.95	4.85	5.25	6.40		5.32	5.42

Table XIV. Physical Compatibility and pH of Polynute, Admixed with all Selected Vitamins in the Presence of Calcium, Magnesium, and/or Phosphate.

Polynute with
Calcium 20 mEq/L
Phosphate 20 mEq/L
Folic Acid 5 mg/L
B-12 1000 mcg/L
K-1 10 mg/L
plus:

	Regular Insulin units/L							Heparin Sodium units/L	
	20	40	60	80	100			1000	
M.V.I. 10 ml/L	C	C	C	C	C			C	
pH	5.75	5.75	5.77	5.77	5.77			5.78	
Solu B Forte 10 ml/L	C	C	C	C	C			C	
pH	5.44	5.42	5.42	5.40	5.36			5.45	

Table XV. Physical Compatibility and pH of Polynute, Admixed with all Selected Vitamins in the Presence of Calcium, Phosphate, and Insulin and Heparin.

CHEMICAL COMPATIBILITY

U.V. Spectroscopy

Continuous U.V. absorption spectra were obtained for triplicate additive-Polynute samples. An aliquot was removed from the samples and diluted for scan at one, four, eight, and twenty-four hours after admixture.

In preparation of the references for the calcium-phosphate series with the vitamin additives, it was found that concentrations of electrolytes compatible in the Polynute are incompatible in aqueous solution. The problem was to combine all those additives found in the additive-Polynute sample, in the reference such that the spectrum read by the spectrophotometer would be that of the Polynute only. U.V. absorption was measured for (1) water alone, (2) dextrose and water, and (3) water containing the individual electrolytes (i.e., calcium gluconate and potassium phosphate). No U.V. absorption was observed in any of these three solutions. This would indicate that dextrose and these ions do not absorb U.V. light at those concentrations required for scanning. Therefore, when calcium and phosphate were both present in a test admixture, they were omitted from the aqueous reference solution. This was necessitated by the limited solubility of calcium phosphate in water. This procedure permitted obtaining the absorption spectra of Polynute. The Beer's Law Plot for Polynute and U.V. absorption spectrum of Polynute are shown in Fig. 1 and 2, respectively.

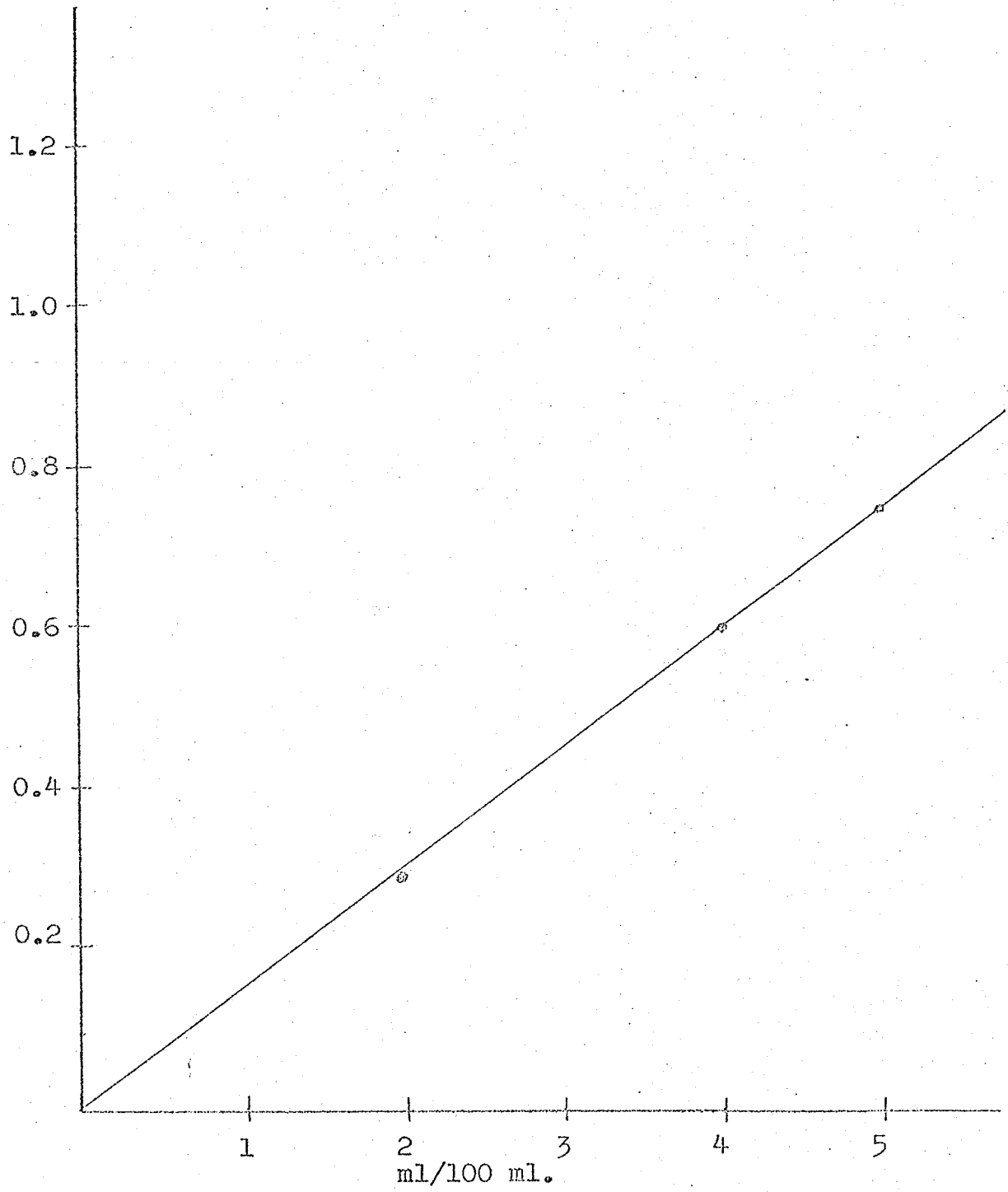

Fig. 1. Beer's Law plot for Polynute

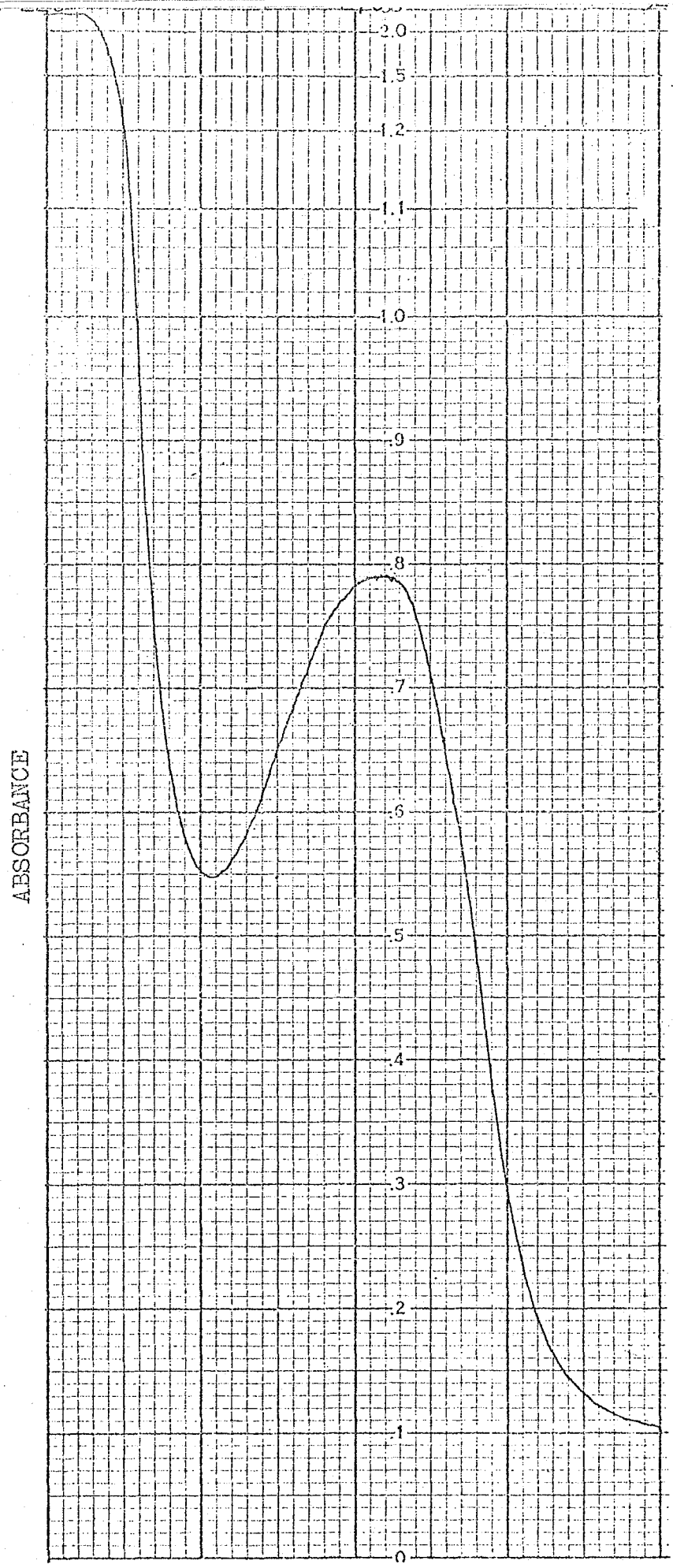

Fig. 2. U.V. Spectrum of Polynute (λ max 276) at 5 ml/100 ml

Phytonadione, Folic Acid, and Cyanocobalamin

The preliminary study was conducted in the following manner. Each vitamin was admixed with each electrolyte singly, and in combination (calcium and phosphate) at a therapeutic concentration and a maximum compatible concentration. These combinations of references and samples were prepared:

I. Reference: water and phytonadione 10 mg/L
Sample: Polynute, phytonadione 10 mg/L, calcium 20 mEq/L, and phosphate 20 mEq/L

II. Reference: water and folic acid 5 mg/L
Sample: Polynute, folic acid 5 mg/L, calcium 20 mEq/L, and phosphate 20 mEq/L

III. Reference: water and cyanocobalamin 1000 mcg/L
Sample: Polynute, cyanocobalamin 1000 mcg/L, calcium 20 mEq/L, and phosphate 20 mEq/L

If there were no alterations in spectra, then that additive was considered apparently compatible with the Polynute. This was true with phytonadione (Fig. 3), folic acid (Fig. 4), and cyanocobalamin (Fig. 5).

These scans represent samples at intervals of one and eight hours after admixture with potassium phosphate 20 mEq/L and calcium gluconate 20 mEq/L.

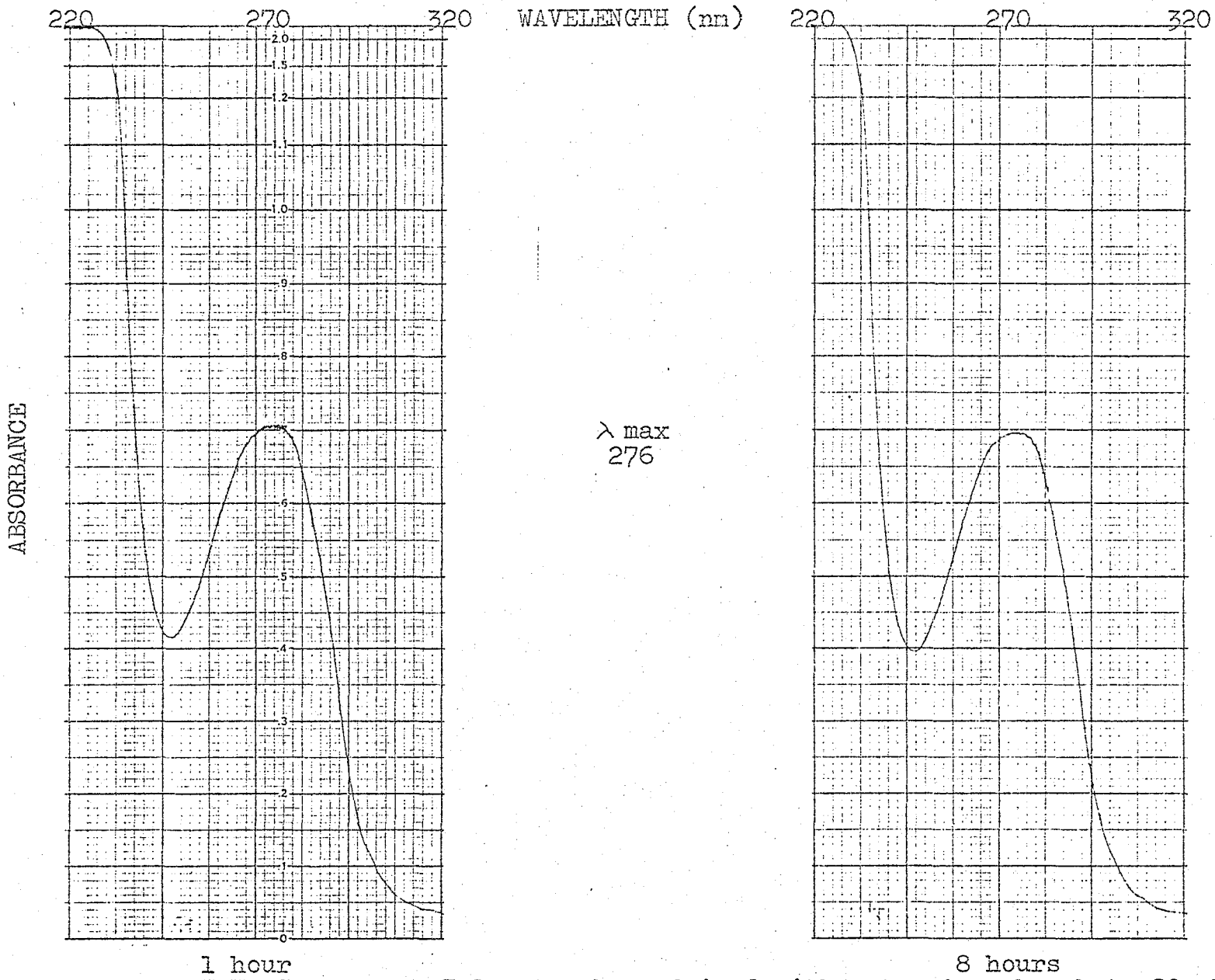

Fig. 3. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and phytonadione 10 mg/L.

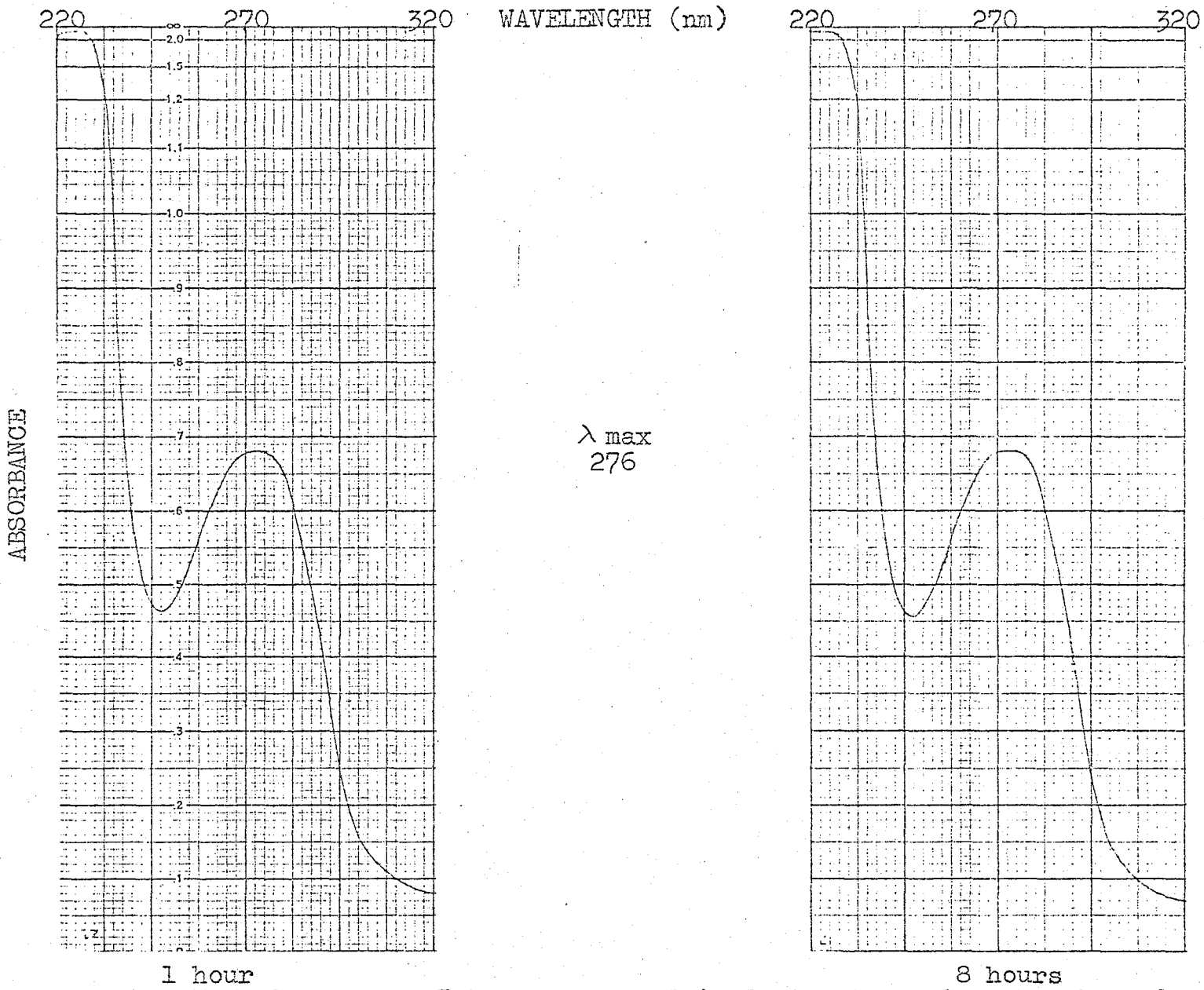

Fig. 4. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium fluconate 20 mEq/L, and folic acid 5 mg/L.

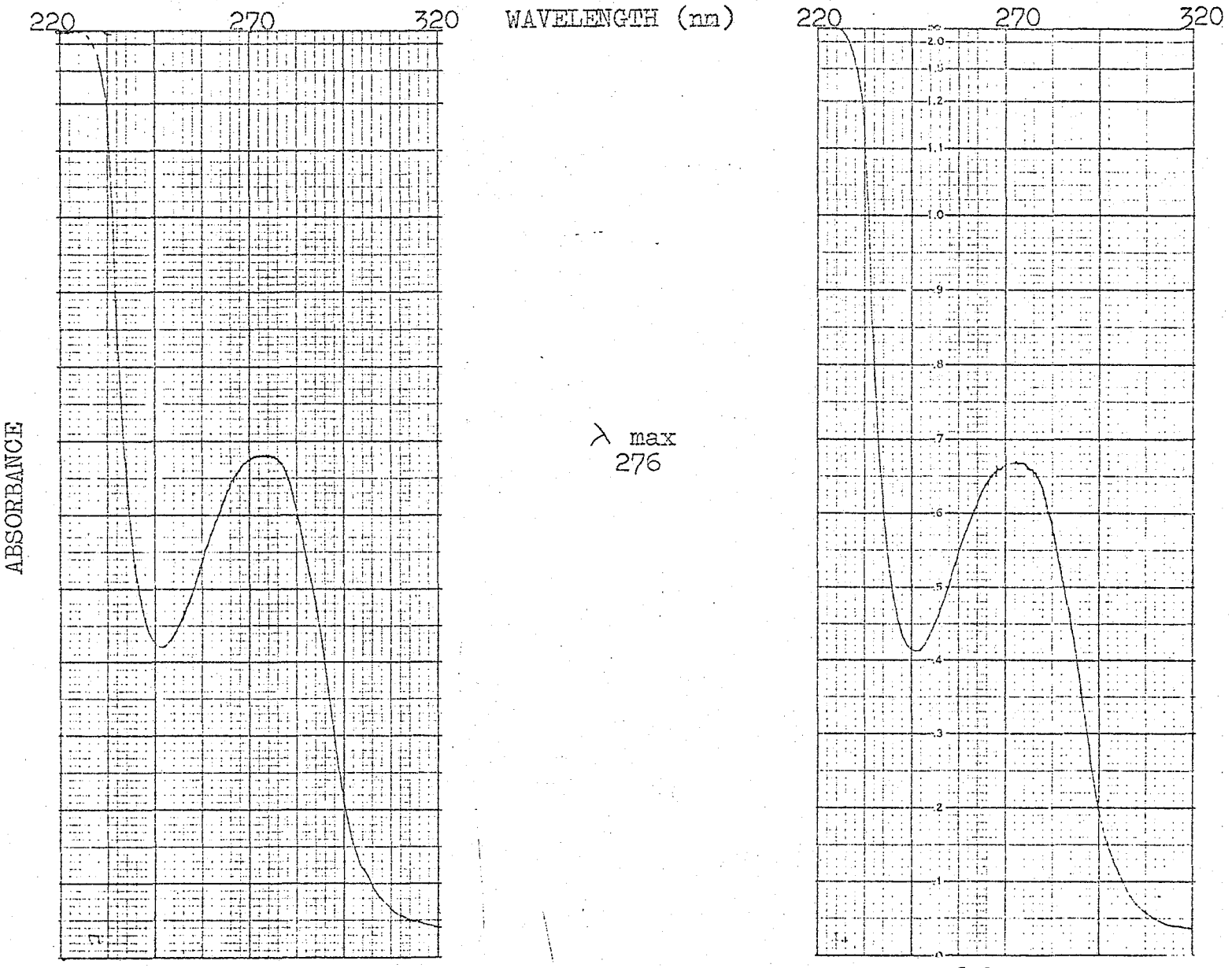

Fig. 5. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and cyanocobalamin 1000 mcg/L.

M.V.I.

The following combinations of references and samples were prepared for M.V.I., 10 ml/L:

I. Reference: water and M.V.I.
Sample: Polynute, M.V.I., calcium 20 mEq/L, and phosphate 20 mEq/L

II. Reference: water and M.V.I.
Sample: Polynute, M.V.I., calcium 5 mEq/L, and phosphate 10 mEq/L

III. Reference: water, M.V.I., and phosphate 10 mEq/L
Sample: Polynute, M.V.I., and phosphate 10 mEq/L

IV. Reference: water, M.V.I., and calcium 5 mEq/L
Sample: Polynute, M.V.I., and calcium 5 mEq/L

M.V.I. exhibited a hypsochromic shift from 278 nm to 267 nm in the solution admixed with both calcium gluconate, 20 mEq/L, and potassium phosphate, 20 mEq/L (Fig. 6,7). The lowering of the electrolyte concentrations did not alter the observed effect (Fig. 8,9). With potassium phosphate, 10 mEq/L, a second λ max is detectable at 261 nm at the one hour interval (Fig. 10,11). There appears to be a growth of this peak over a time period, which apparently influences the observed shift. This effect is also observable with calcium gluconate, 5 mEq/L (Fig. 12,13). In this set, the shift is more easily detectable at the four hour interval.

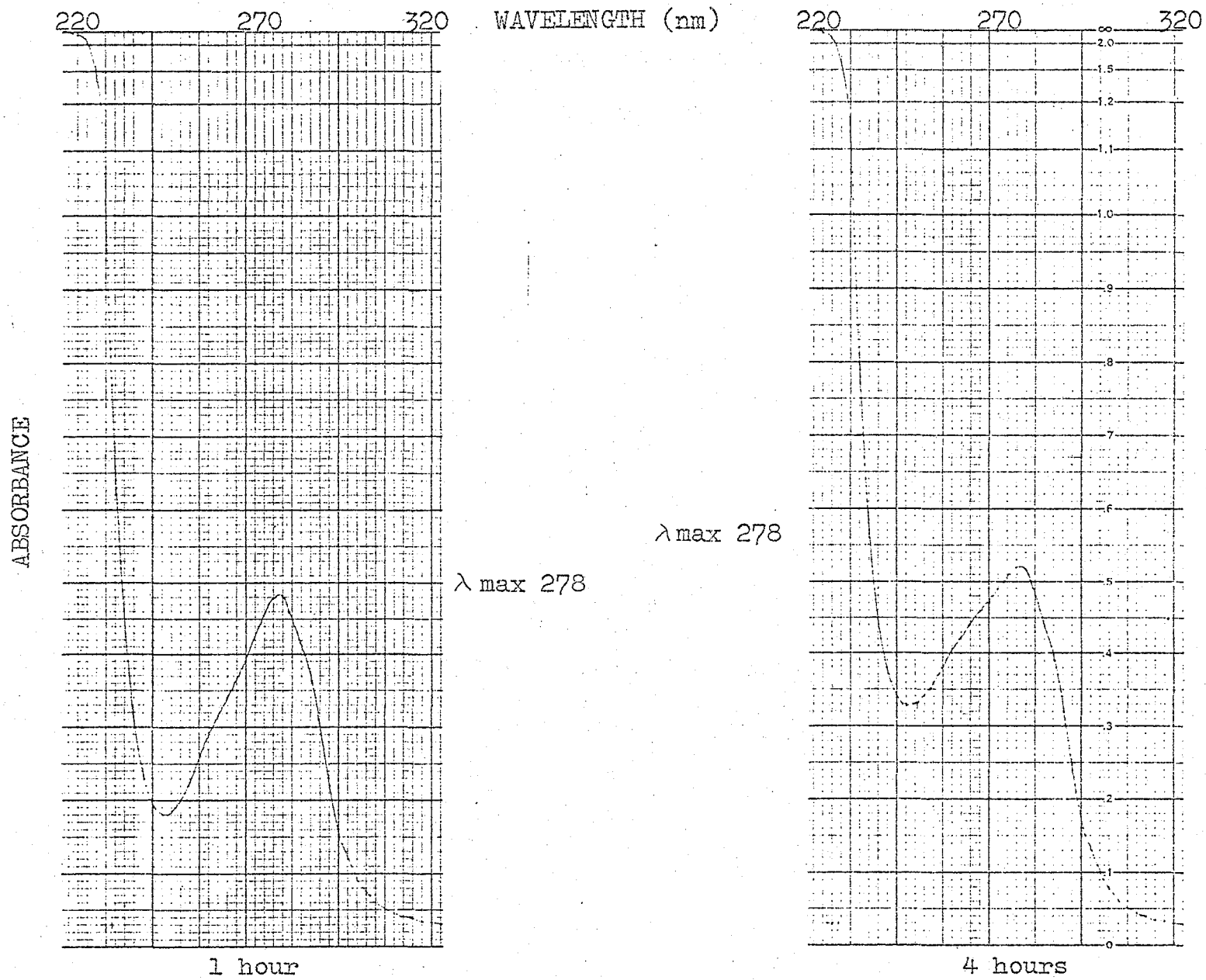

Fig. 6. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and M.V.I. 10 ml/L.

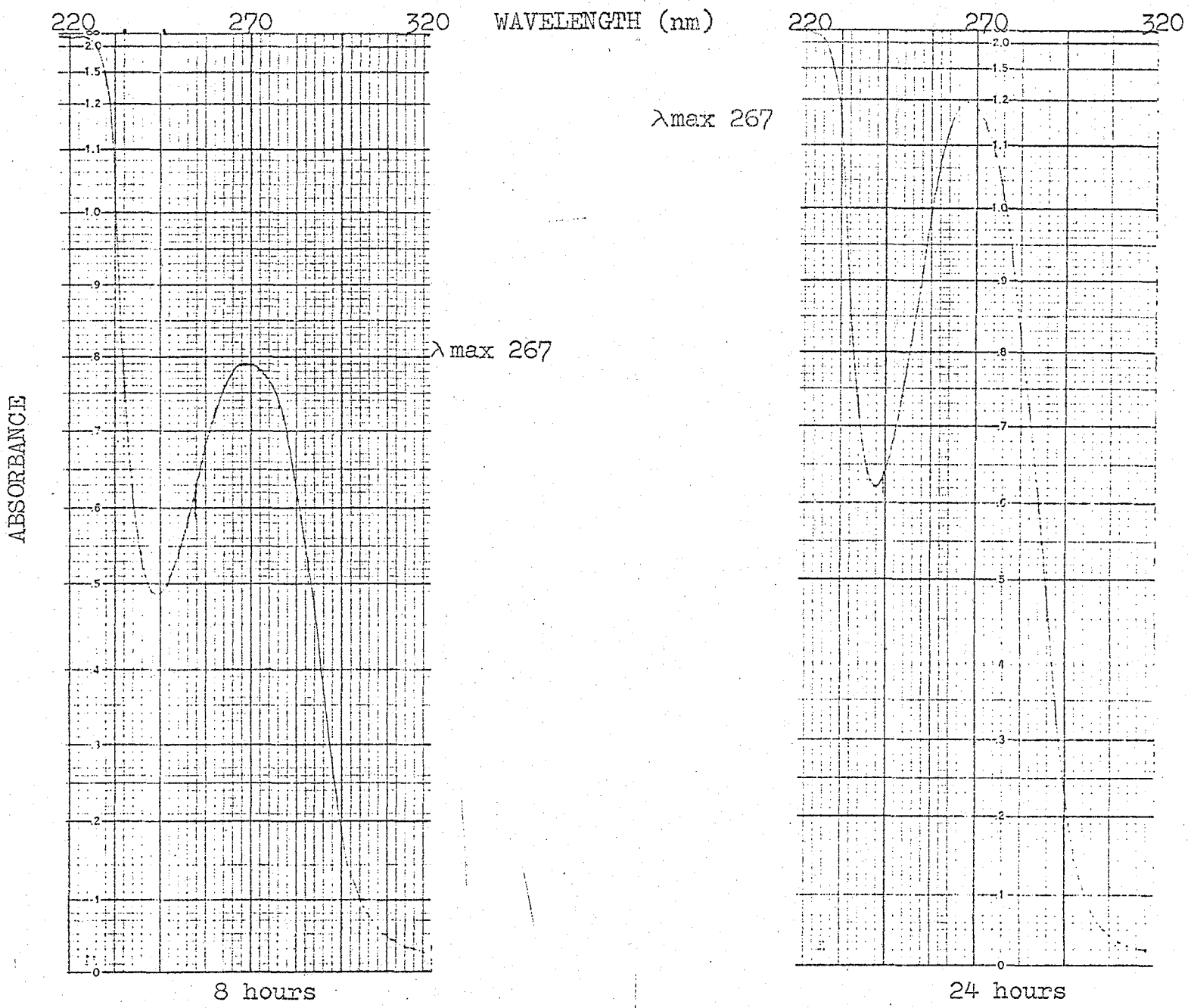

Fig. 7. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and M.V.I. 10 ml/L.

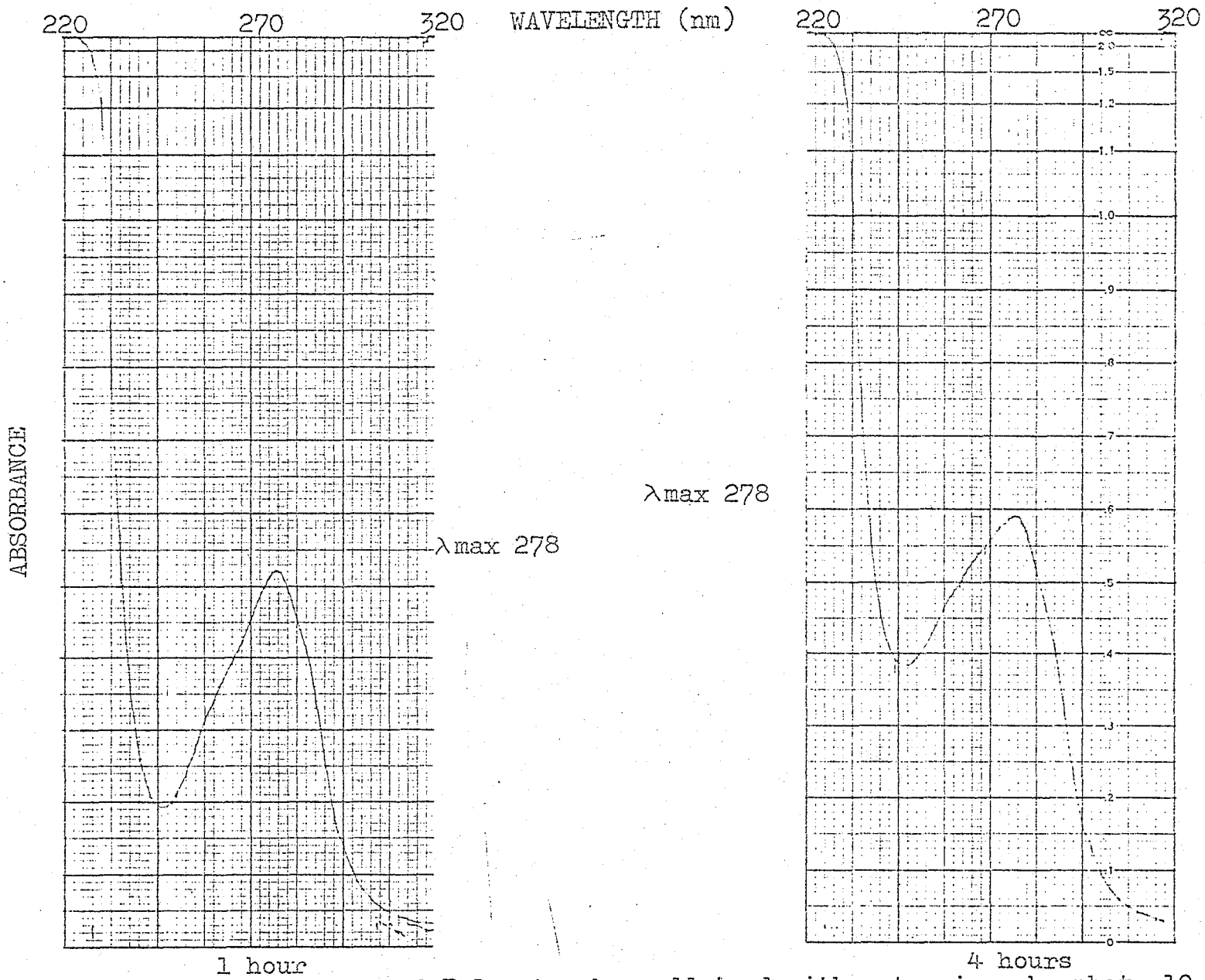

Fig. 8. U.V. Spectra of Polynute when addmixed with potassium phosphate 10 mEq/L, calcium gluconate 5 mEq/L, and M.V.I. 10 ml/L.

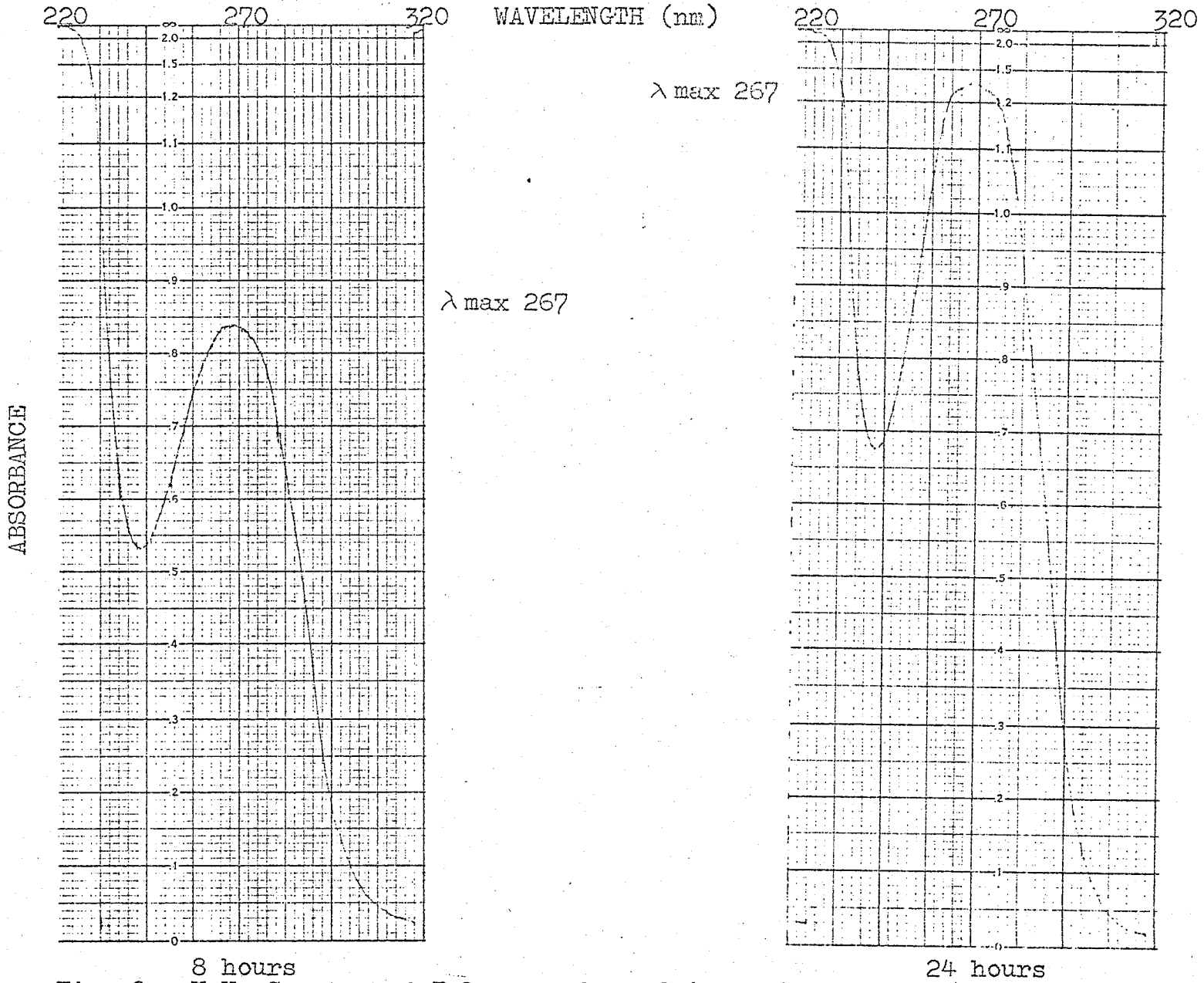

Fig. 9. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L, calcium gluconate 5 mEq/L, and M.V.I. 10 ml/L.

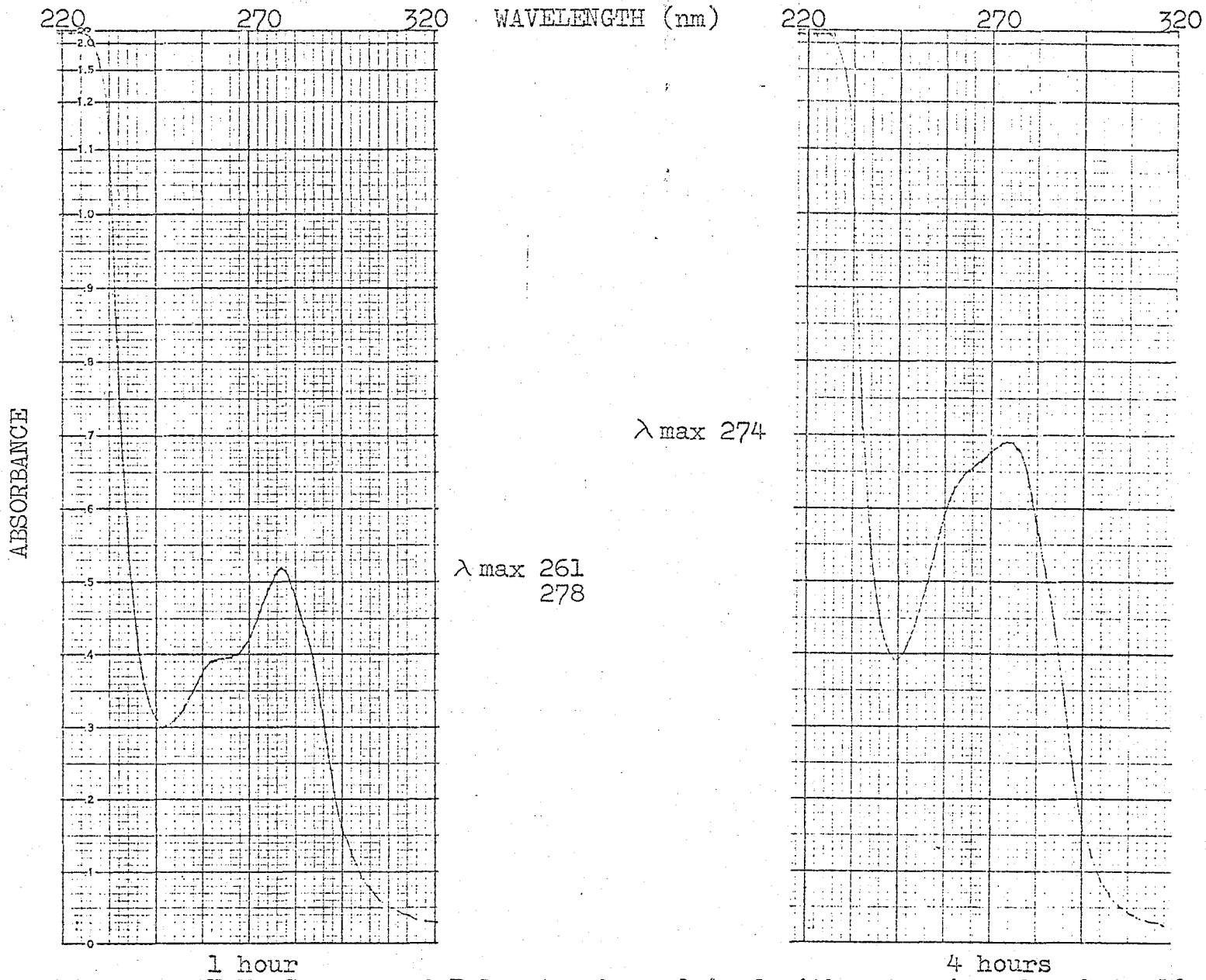

Fig. 10. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L and M.V.I. 10 ml/L.

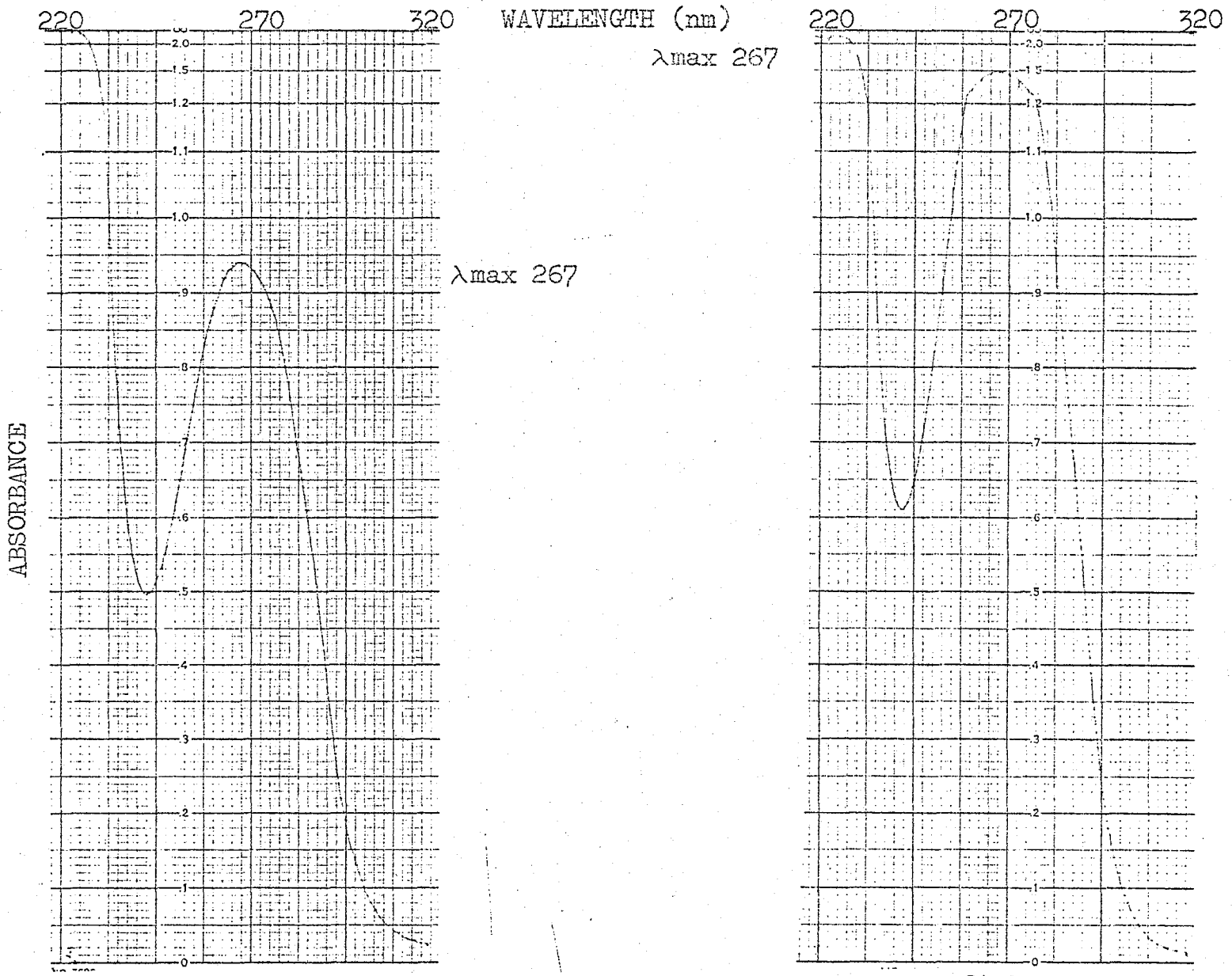

Fig. 11. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L and M.V.I. 10 ml/L.

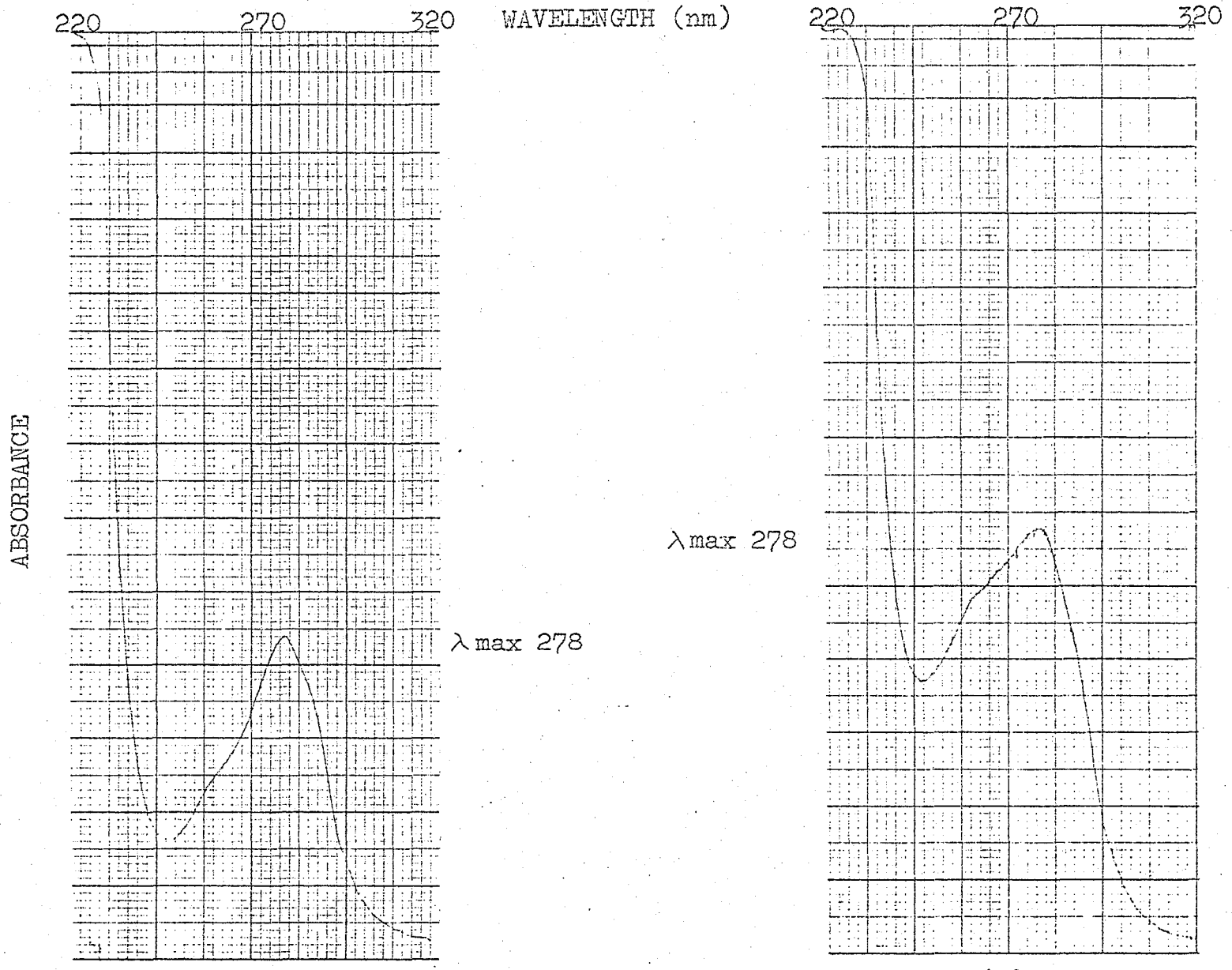

Fig. 12. U.V. Spectra of Polynute when admixed with calcium gluconate 5 mEq/L and M.V.I. 10 ml/L.

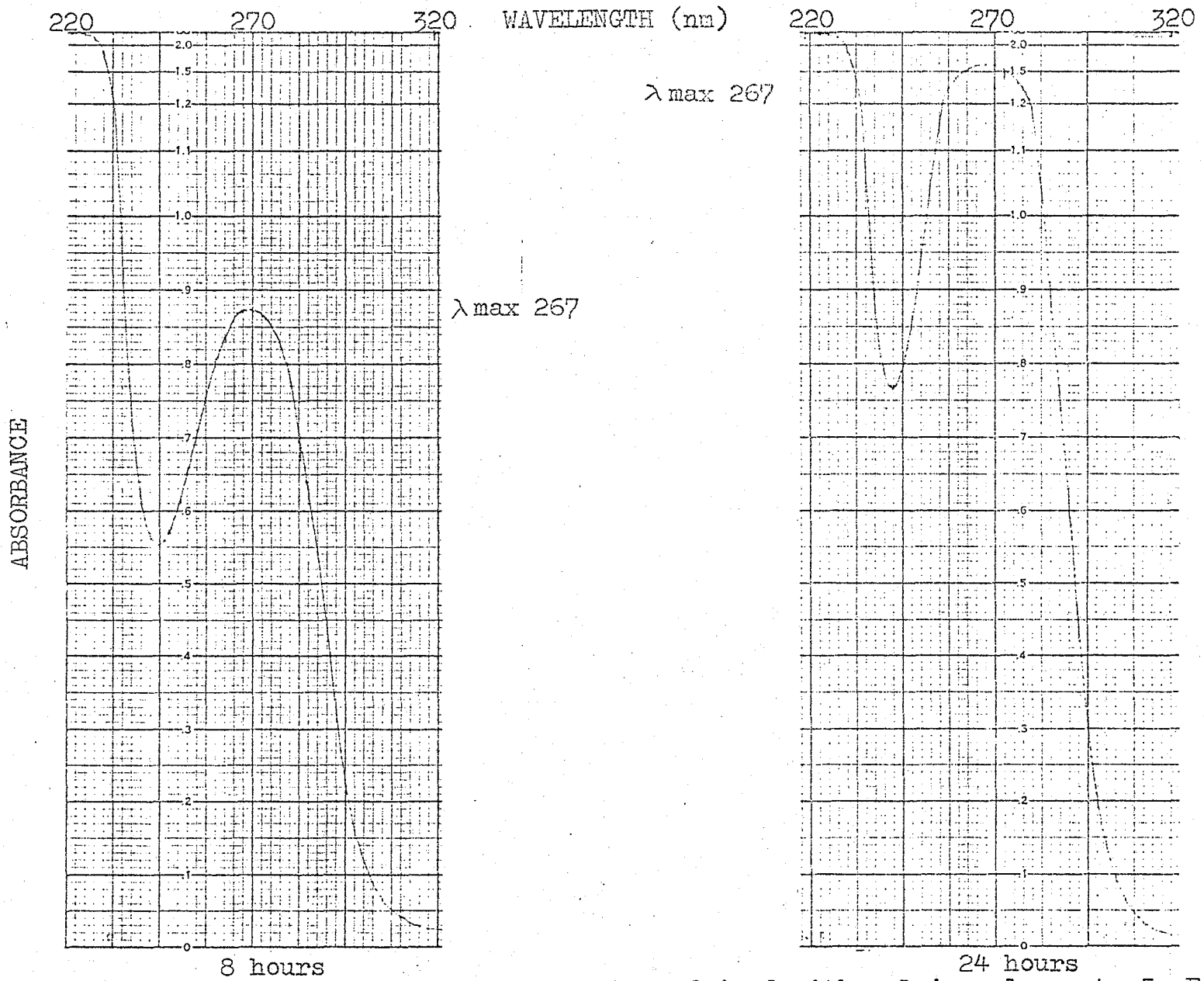

Fig. 13. U.V. Spectra of Polynute when admixed with calcium gluconate 5 mEq/L and M.V.I. 10 ml/L.

Solu B Forte

The following combinations or references and samples were prepared for Solu B Forte, 10 ml/L:

I. Reference: water and Solu B Forte
Sample: Polynute, Solu B Forte, calcium 20 mEq/L, and phosphate 20 mEq/L

II. Reference: water and Solu B Forte
Sample: Polynute, Solu B Forte, calcium 5 mEq/L and phosphate 10 mEq/L

III. Reference: water, Solu B Forte, and phosphate 10 mEq/L
Sample: Polynute, Solu B Forte, and phosphate 10 mEq/L

IV. Reference: water, Solu B Forte, and calcium 5 mEq/L
Sample: Polynute, Solu B Forte, and calcium 5 mEq/L

When Solu B Forte is added to Polynute in the presence of calcium gluconate, 20 mEq/L, and potassium phosphate, 20 mEq/L (Set I), a three-peaked spectrum is obtained (Fig. 14,15). There is little change from the one to the four hour interval, but at the eight hour interval, a hypsochromic shift is evident. At lower electrolyte concentrations, the 231 nm peak is more pronounced at the one hour interval (Fig. 16, 17). The shift to shorter wavelengths is again evident. When in the presence of potassium phosphate, 10 mEq/L, alone, there is a change of spectra (Fig. 18). This two peaked scan has no peak at the 251 nm observed in those scans with both electrolytes. At the four hour interval, the 251 nm peak becomes more evident. The absorbance increases with time until it surpassed the 232 nm peak at 24 hours (Fig. 19). This does not develop with calcium gluconate, 5 mEq/L, as its spectrum is very similar to those with both electrolytes (Fig. 20,21).

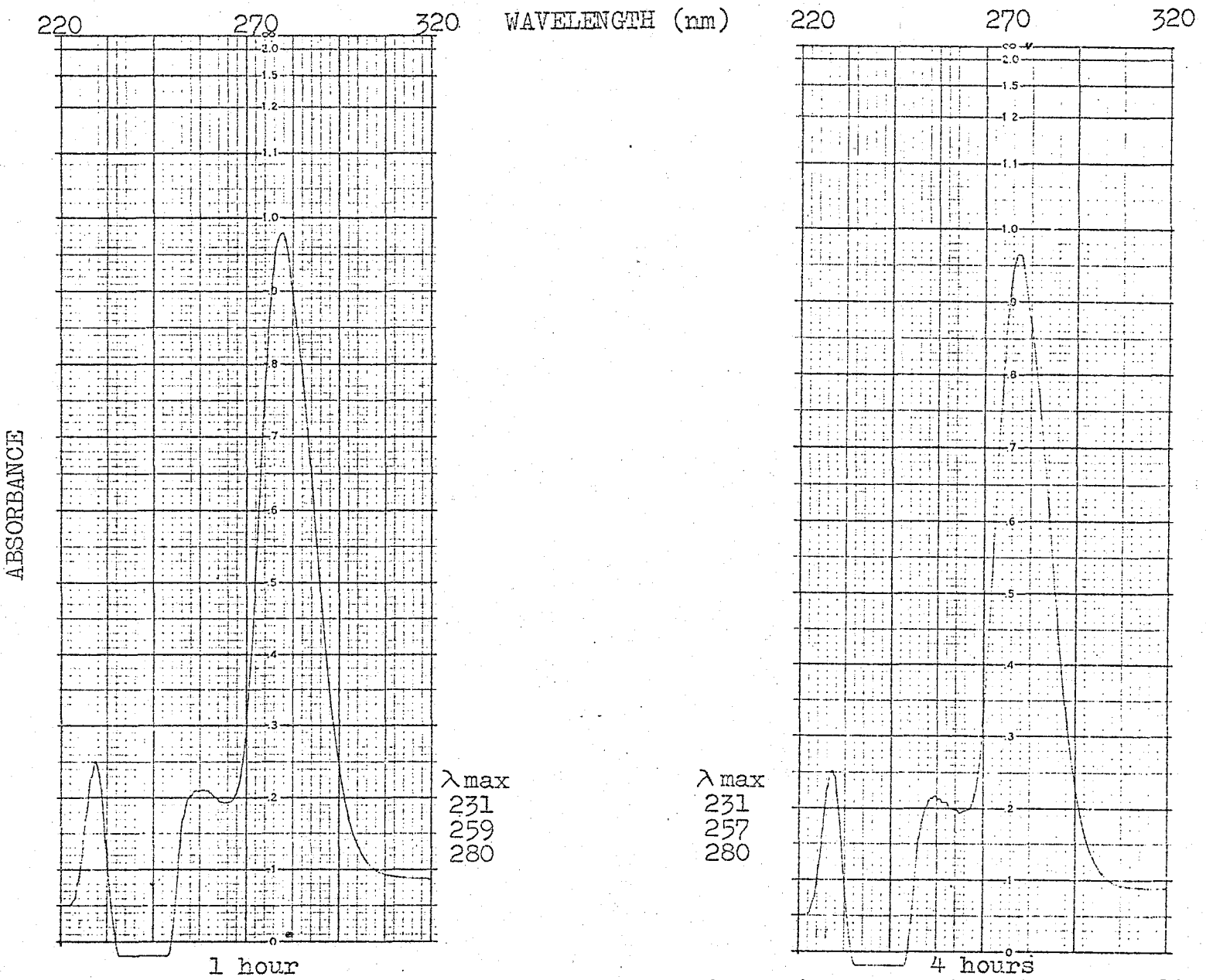

Fig. 14. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and Solu B Forte 10 ml/L.

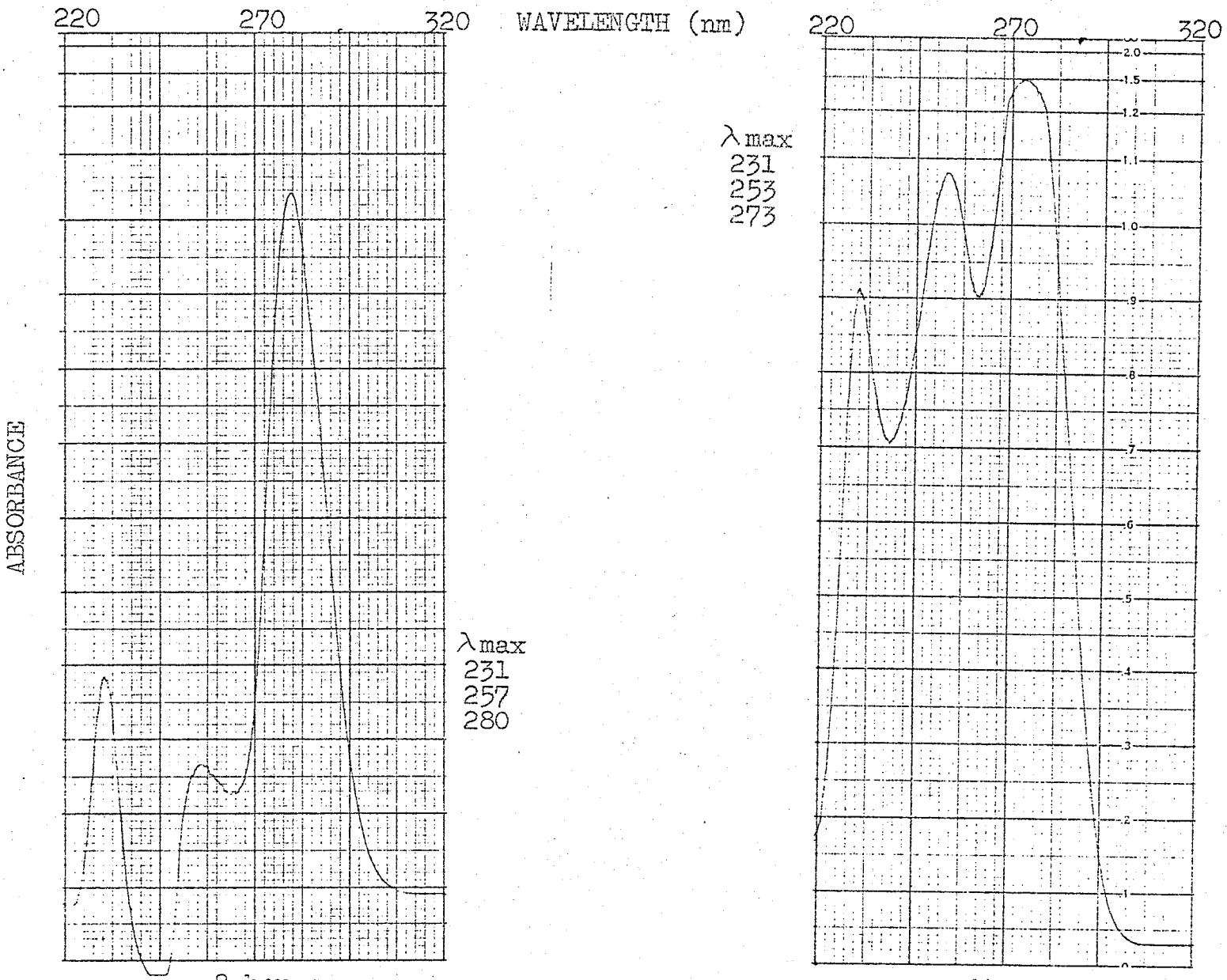

Fig. 15. U.V. Spectra of Polynute when admixed with potassium phosphate 20 mEq/L, calcium gluconate 20 mEq/L, and Solu B Forte 10 ml/L.

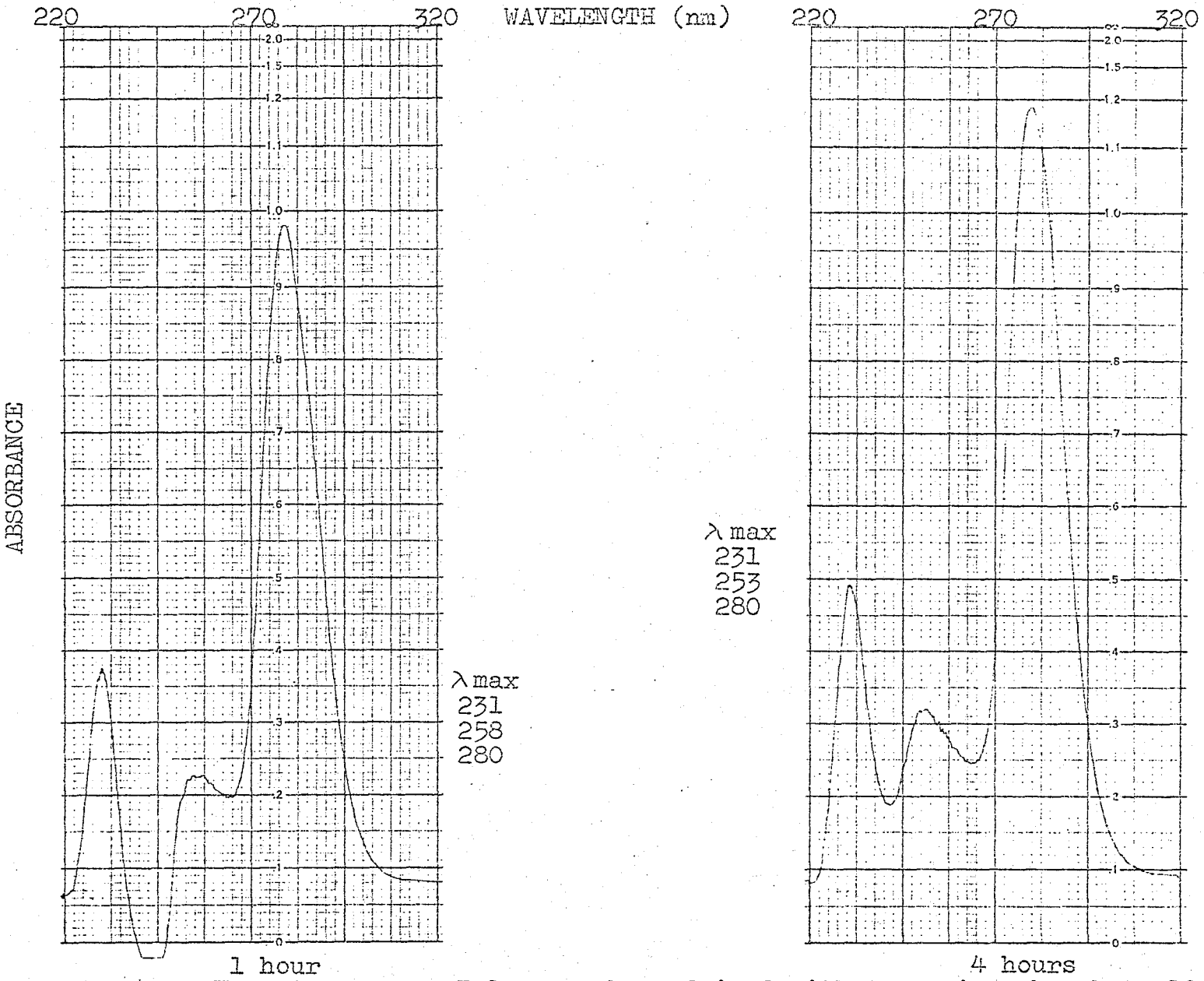

Fig. 16. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L, calcium gluconate 5 mEq/L, and Solu B Forte 10 ml/L.

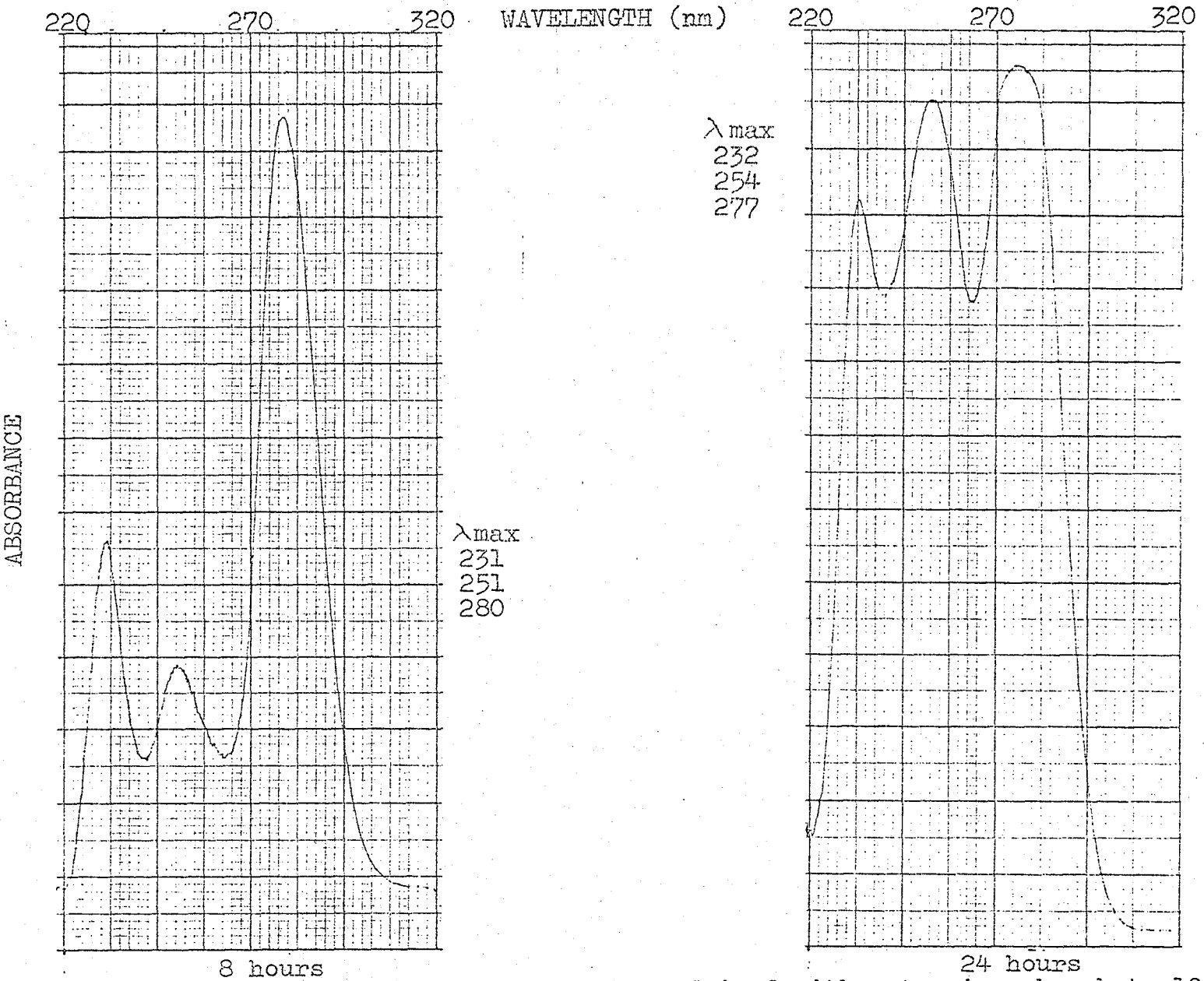

Fig. 17. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L, calcium gluconate 5 mEq/L, and Solu B Forte 10 ml/L.

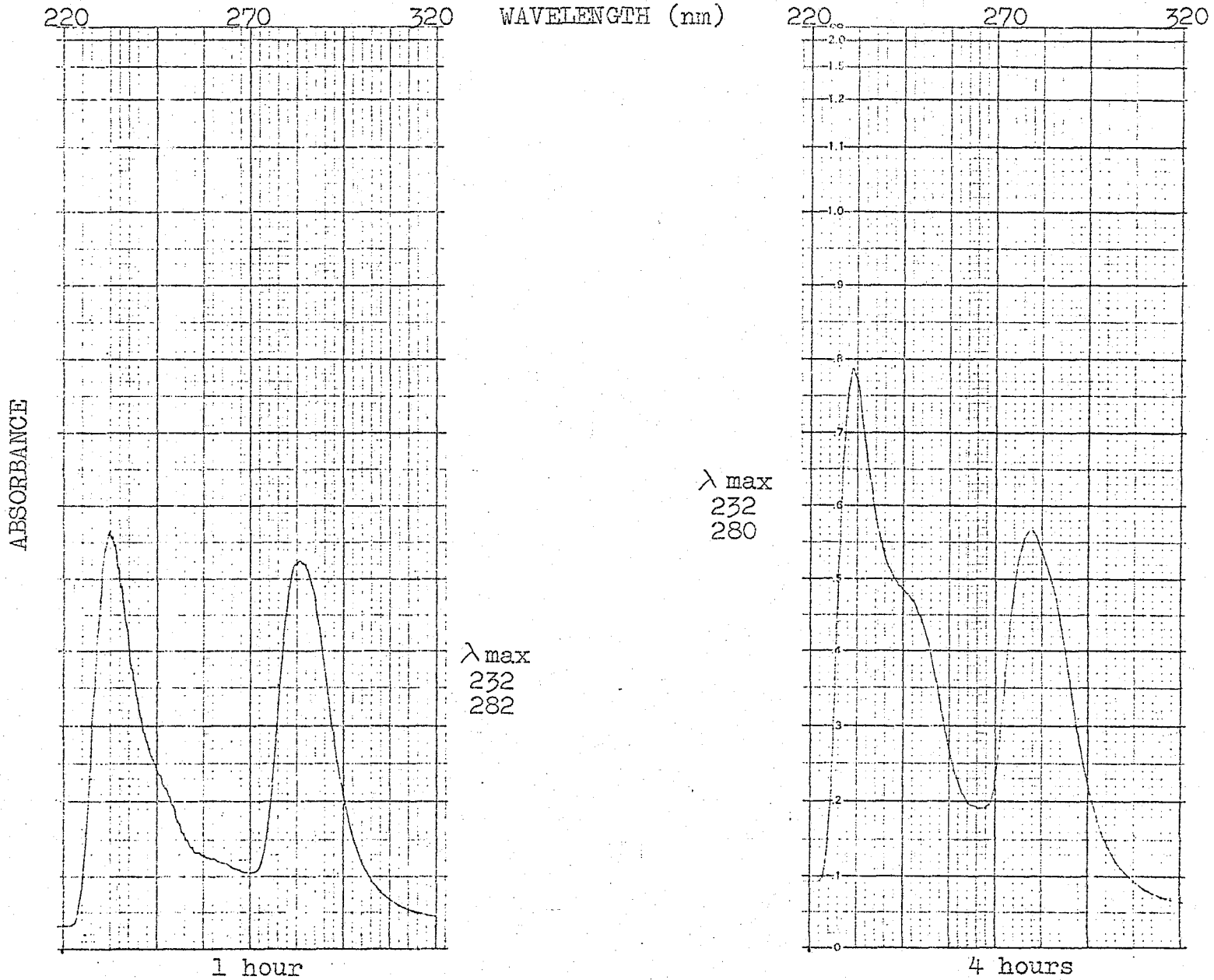

Fig. 18. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L and Solu B Forte 10 ml/L.

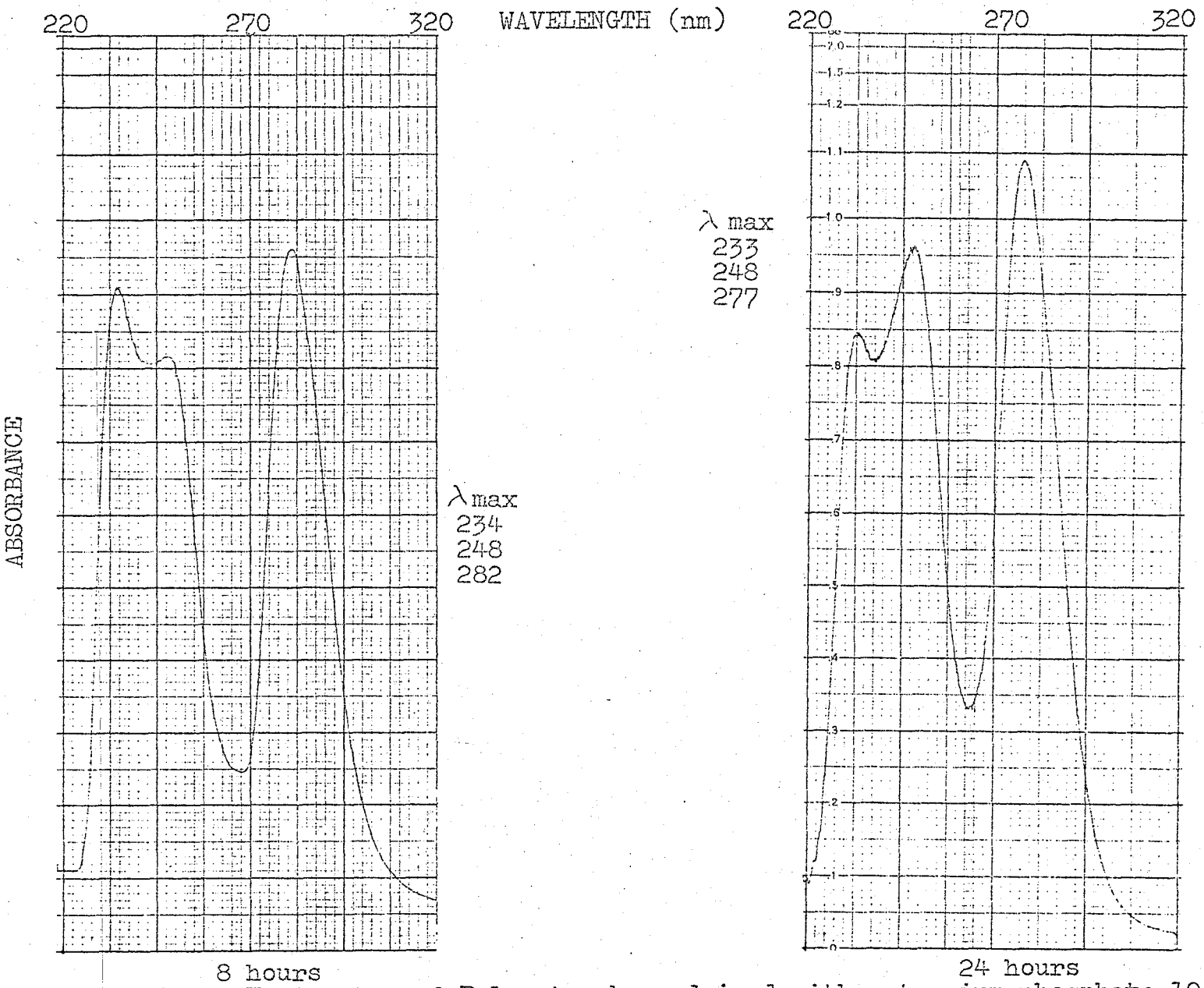

Fig. 19. U.V. Spectra of Polynute when admixed with potassium phosphate 10 mEq/L and Solu B Forte 10 ml/L.

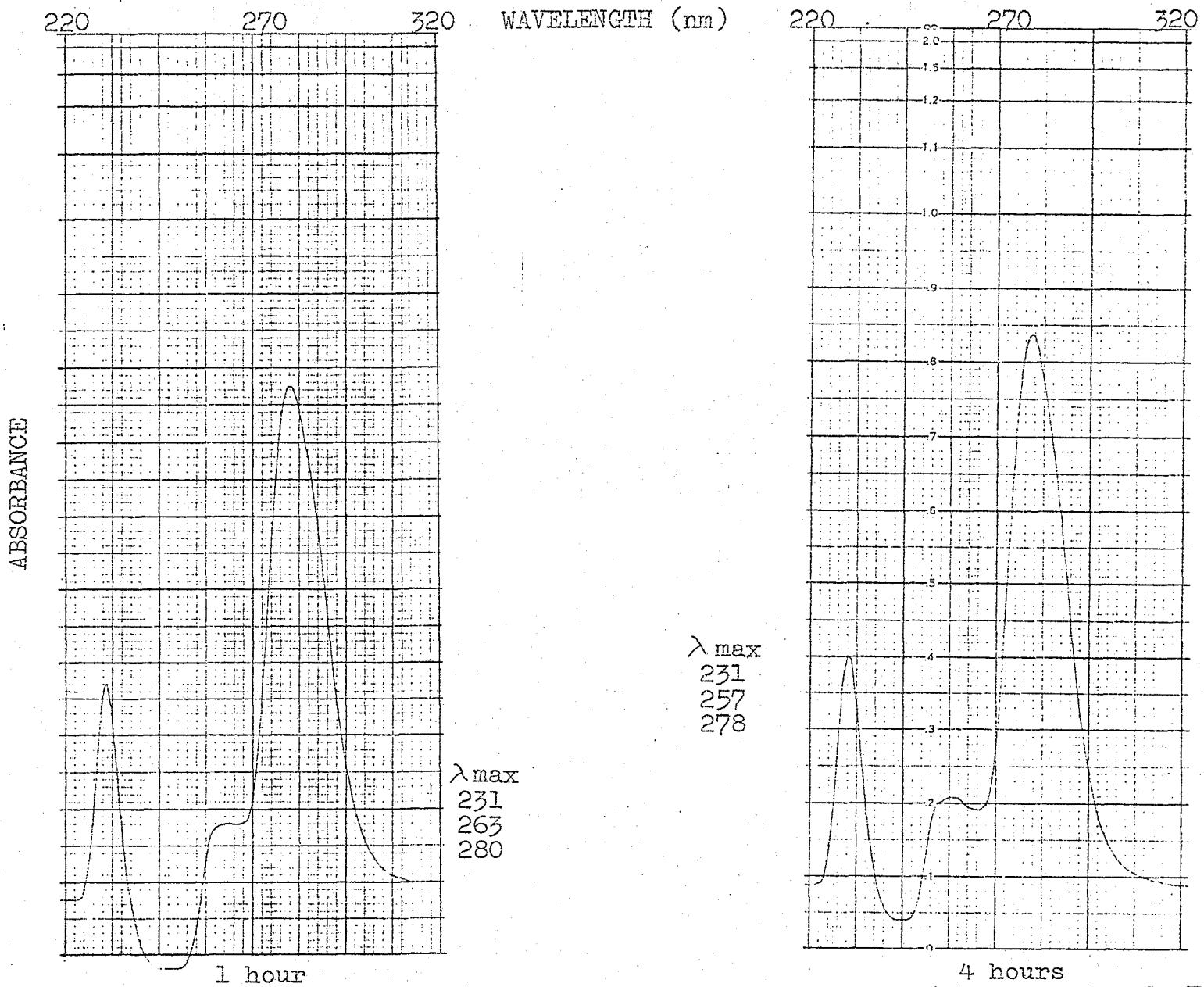

Fig. 20. U.V. Spectra of Polynute when admixed with Calcium gluconate 5 mEq/L and Solu B Forte 10 ml/L.

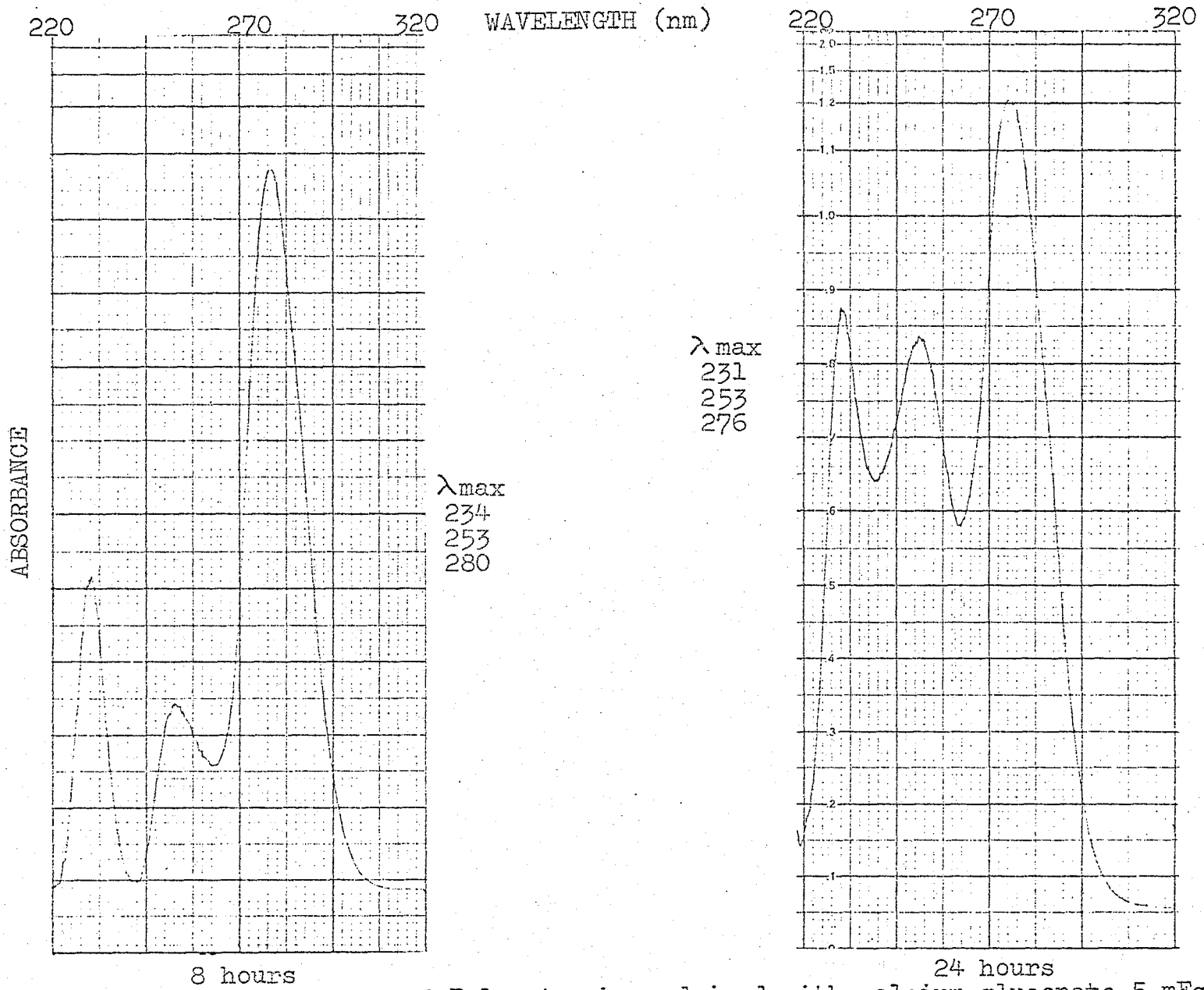

Fig. 21. U.V. Spectra of Polynute when admixed with calcium gluconate 5 mEq/L and Solu B Forte 10 ml/L.

Thin-Layer Chromatography

Thin-Layer Chromatography (TLC) analysis was conducted with duplicate samples, each prepared every eight hours to facilitate running a sample and a control plate containing Polynute only, simultaneously. Due to the two plate capacity of the DeSaga-Heidelberg developing tanks, only one sample-control set could be developed at each interval. The four hour interval after admixture was chosen as the sample time for the TLC analysis.

Of the combinations that exhibited spectral changes, samples from the following mixtures were examined:

I. Polynute when admixed with calcium gluconate 5 mEq/L and Solu B Forte 10 ml/L

II. Polynute when admixed with calcium gluconate 5 mEq/L and M.V.I. 10 ml/L

III. Polynute when admixed with potassium phosphate 10 mEq/L and Solu B Forte 10 ml/L

IV. Polynute when admixed with potassium phosphate 10 mEq/L and M.V.I. 10 ml/L

V. Polynute when admixed with calcium gluconate 20 mEq/L, potassium phosphate 20 mEq/L, and Solu B Forte 10 ml/L

VI. Polynute when admixed with calcium gluconate 20 mEq/L, potassium phosphate 20 mEq/L, and M.V.I. 10 ml/L

Single plates of the following mixtures were also analyzed:

VII. Polynute when admixed with calcium 20 mEq/L, phosphate 20 mEq/L, folic acid 5 mg/L, cyanocobalamin 1000 mcg/L, phytonadione 10 mg/L, and Solu B Forte 10 ml/L

VIII. Polynute when admixed with calcium 20 mEq/L, phosphate 20 mEq/L, folic acid 5 mg/L, cyanocobalamin 1000 mcg/L, phytonadione 10 mg/L, and M.V.I. 10 ml/L

Each additive-Polynute combination resulted in the same

chromatographic pattern observed in Figure 22. The control and sample chromatograms could not be differentiated visually. Examination of the TLC plates under ultraviolet light did not reveal any fluorescent products.

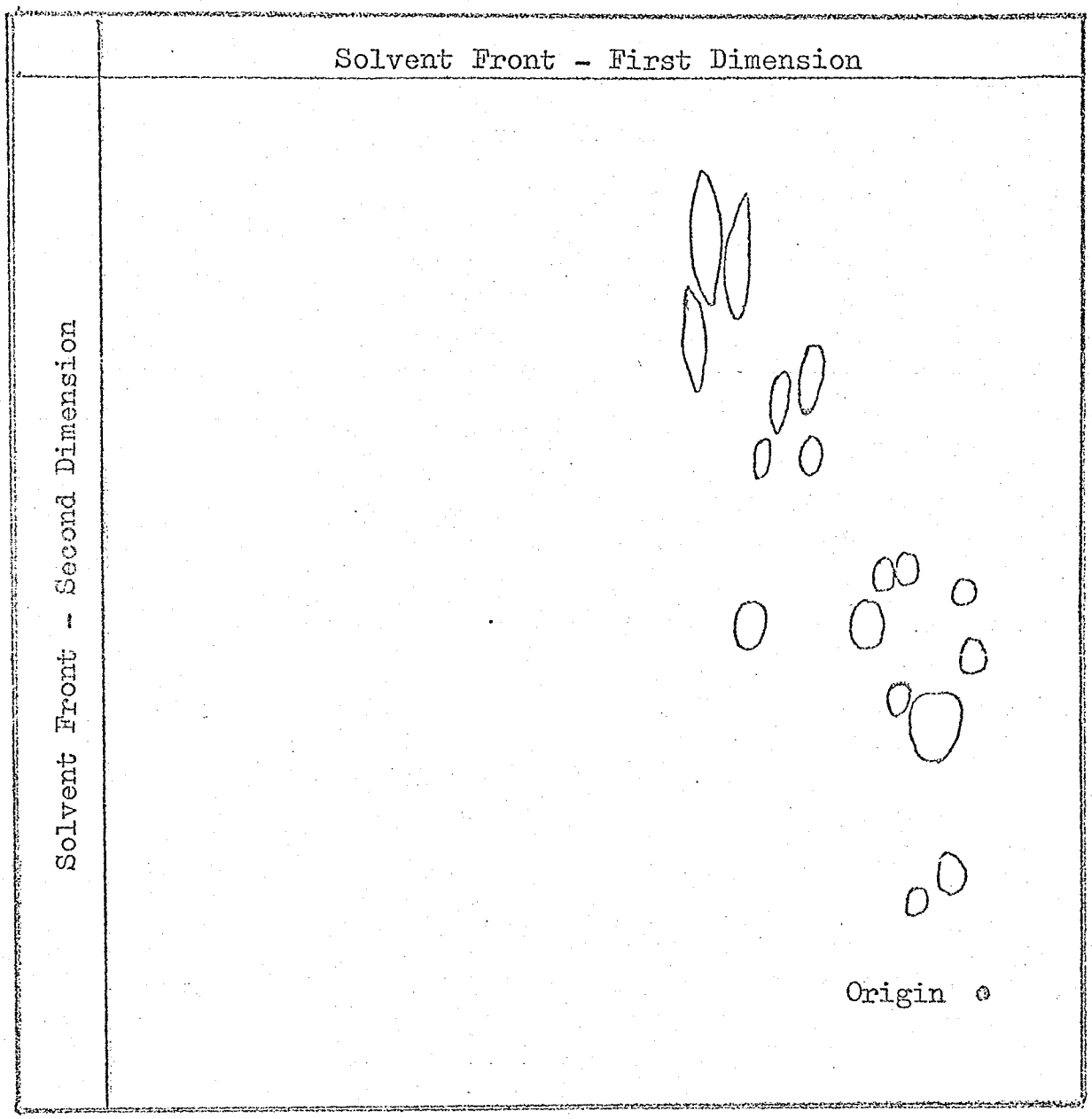

Fig. 22. TLC plate spotted with 0.5 microliters of Polynute, after development with 0.2% ninhydrin spray.

IV. DISCUSSION

It has been noted that the pH of the parenteral solution is an important factor in the compatibility of admixing essential additives. The pH of the additive, its concentration, and the pH of the basic parenteral nutrition solution all contribute to the final pH. The presence of a physical incompatibility can also alter the pH of the solution (47) which could also affect other additives present. Certainly, the stability of various common additives at the pH(5.3) of Polynute must always be considered.

The compatibility of calcium gluconate and potassium phosphate in protein hydrolysate is affected by the order of mixing. This effect can be seen in the maximum compatible concentration obtained with each sequence. Although a complete study was not performed on the effect of order of mixing on these two ions, a trend could be distinguished. Collin and co-workers (52) have studied the compatibility of calcium gluceptate and potassium phosphate in 8% amino acid solution (FreAmine[a]) and have made specific recommendations. They report the calcium and phosphate ions should be added to the 50% dextrose and FreAmine units respectively, prior to their mixing, or that calcium ion be added as the

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a - McGaw Laboratories, Glendale, Ca.

last electrolyte to the FreAmine-dextrose solution.

Certain drug previously reported as incompatible with protein hydrolysates (48,49,50) were found to be physically compatible in this study. Among these were heparin sodium, regular insulin, magnesium sulfate, cyanocobalamin, and phytonadione.

The electrolyte concentrations already present in the protein hydrolysate should always be considered when preparing a parenteral nutrition solution in the clinical setting. In addition to the concentrations added in this study, Polynute contains the following concentrations of the selected electrolytes: calcium 4 mEq/L, magnesium 1 mEq/L, and phosphate 8 mEq/L.

The evaluation of the U.V. spectra was based on alteration in the scan, which is apparently due to a chemical interaction. A decrease or increase at the absorption maximum (λ max), shift of λ max, or absence or presence of a secondary peak(s) were noted in the results. A decrease or increase at λ max would be indicative of a decrease or increase, respectively, of concentration. A shift of λmax and absence or presence of secondary peaks would be indicative of a change in molecular or electron configuration of the formulation. Because of the experimental design employed, when both calcium and phosphate were required for the reference, the results obtained may actually be a composite of both Polynute and the two electrolytes. However, both calcium and phosphate alone do not absorb U.V. light at the concentrations used in the

scans.

Blackburn (55) reports that the majority of amino acids show no absorption over the range 250 to 320 nm, and phenylalanine, tyrosine, and tryptophan together are responsible for the U.V. absorption of the protein solution. Since tyrosine and tryptophan absorb to such a great degree, it has been reported that absorption due to phenylalanine can be neglected. Rao (57) reports that the absorption of these amino acids occurs at about 260 to 280 nm. Goodwin and Morton (54) pointed out that protein hydrolysates can be treated as two-component systems for analysis by spectrophotometry. They assume that neither the presence of the other amino acids nor the mode of the linkages significantly distorts or displaces the tyrosine-tryptophan absorption. Coulter and co-workers (56) concluded that there is no displacement due to the peptide linkage. However, Rao (57) reports some absorption from 200 to 240 nm.

The optimum concentration required for continuous U.V. absorption spectra for folic acid, cyanocobalamin, and phytonadione was too high to be considered therapeutic and, therefore, was not examined. However, at therapeutic concentrations, there was no apparent effect on the Polynute spectra.

Due to the limitations of the spectrophotometer, dilutions of the admixed solutions were required to obtain the U.V. scans. If a chemical interaction were to be concentration dependent, then this may have affected its detection.

One must also consider the possibility of such concentration dependent interaction as being reversible or irreversible.

Those additive-Polynute combinations that exhibited apparent changes in the U.V. analysis were prepared again and spotted on TLC plates, four hours after admixture. This was done to determine if the altered spectra were due to a demonstrable chemical interaction or change. The TLC technique employed provides information on 17 of the amino acids and peptides present in the protein hydrolysate. Nothing can be said about the chemical integrity of the other additives, the remaining amino acids, and/or the peptides.

Solu B Forte and M.V.I. were admixed with potassium phosphate and calcium gluconate at a concentration of 20 mEq/L each. There was no visible change in the chromatogram or Rf values. Both vitamin additives were added to the electrolytes individually and this resulted in no change. The addition of all the additives in concentrations used in the physical study (Table 17) did not alter the results.

On the basis of the data presented here, it appears that the major compatibility problem in protein hydrolysate-dextrose solutions will be that of high concentrations of calcium and phosphate ion. The compatible concentration ranges for these electrolytes has been mapped in Table VI. If a precipitation of calcium phosphate is to occur, it will be manifested almost immediately after admixture of the components. It has been demonstrated that greater concentrations of these ions will be tolerated by Polynute if the calcium component

is added last (see Table VIII). The reason for this phenomenon remains unclear. The maximum compatible concentrations does not seem to be affected by the addition of the other drugs included in this work. Common additives such as vitamins, insulin, heparin, and magnesium have not been shown to produce physical incompatibilities, in usual therapeutic concentrations.

Although alterations in U.V. absorption spectra of Polynute were seen when in the presence of M.V.I. (see Fig. 6 to 13) and Solu B Forte (see Fig. 14 to 21), there was no confirmation of this chemical interaction by TLC.

V. SUMMARY AND CONCLUSION

In total patient care, there is a definite relationship between proper nutirition and maintenance of health. Certainly the role of proteins, calories, vitamins, and minerals have been well documented. Their importance is magnified in instances of trauma and infection. Parenteral nutrition has developed into a practical method of feeding patients unable to take an adequate diet. Yet, if in an attempt to provide all these essential nutrients parenterally, there is some physico-chemical interaction which destroys the biological effectiveness of an ingredient, then another method of administration must be initiated.

The study of the electrolyte additives indicates that admixture of calcium gluconate and potassium phosphate in combination does reach a maximum compatible concentration at about 20 mEq/L of each ion. As a rule, the addition of calcium ion last in the preparation of a hyperalimentation solution should prevent problems, providing concentrations do not exceed those normally considered therapeutic. The addition of calcium gluconate last has resulted in an increase of the maximum compatible concentration to 40 mEq/L of each ion.

The alteration of U.V. spectra indicated that vitamin B complex with C (Solu B Forte) and multiple vitamin infusion

(M.V.I.) may be chemically incompatible with Polynute. This conclusion was not substantiated by thin-layer chromatography. Additional studies will have to be performed to elucidate this point further. Under the conditions set forth in this study, it appears that folic acid, phytonadione, cyanocobalamin, vitamin B complex with C, and multiple vitamin infusion are compatible with a mixture of 590 ml 7% Protein Hydrolysate and 410 ml 50% Dextrose (Polynute) in the presence of limited concentrations of calcium and phosphate.

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