Excipient	Initial %(w/w)	1 Month 40 °C/75% RH %(w/w)	1 Month 50 °C %(w/w)
Carboxymethyl cellulose Na (CMC Na)	96.8	101.6	99.5
Starch 1500	103.3	90.6	95.2
Kollidon SR	81.2 100.2*	99.8	100.9
Polyethyleneoxide N3 03 (PEO)	81.0 99.1*	99.2	99.0
Dibasic Calcium Phosphate	98.4	102.9	102.2
Sodium Stearyl Fumarate	98.9	101.1	103.7
Magnesium Stearate	100.1	101.4	99.3
Colloidal Silicon Dioxide (Cab-o-sil)	99.0	99.4	104.5
Microcrystalline Cellulose	96.8	98.6	107.0
Lactose Monohydrate	94.5	99.2	103.0
Mannitol	111.7	98.1	81.3
Diazoxide Control	N/A	99.0	99.4

* Re-assayed

[00609] Initial low results for ethylcellulose, Kollidon SR, and polyethyleneoxide were investigated by re-preparing samples of diazoxide choline salt and excipient. As shown in Table 6, initial sample recovery (i.e., column "Recovery") of the re-prepared samples indicates method accuracy.

[00610] No reportable impurities were detected in any sample, and no degradation was observed in the samples stored for one-month with the exception of mannitol wherein 81.3% of diazoxide was recovered after 1-month at 50 °C.

3.4.3. Stability Studies for Diazoxide Choline Controlled Release Tablets

[00611] Studies to determine the stability of formulations of diazoxide choline salt as the controlled release tablet were conducted on sample formulations as described in Table 2, results of which are shown in Table 7. In these studies, storage conditions were the following: a) 25 °C/60% RH, and b) 40 °C/75% RH. In Table 7, the term "Appearance" refers to the physical appearance of the tablets of the study. The term "Assay (%)" refers to the percentage of diazoxide choline salt assayed with respect to nominal (i.e., 50 mg or 200 mg) content of the sampleThe term "Dissolution (%)" refers to the amount, expressed as a percentage, of diazoxide choline salt assayed by method described herein.

Table 7. Stability Study for Formulations of Diazoxide Choline Salt.

Test	Time point		
	Initial	1 month	2 months
50 mg tablet, Formulation J, 25 °C/60% RH,			
Appearance	White tablets	White tablets	White tablets
Assay (%)	95.8	102.9	99.3
Dissolution (%):			
3 hr	24	19	15
6 hr	41	30	27
12 hr	62	48	52
24 hr	92	90	94
50 mg tablet, Formulation J, 40 °C/75% RH,			
Appearance	White tablets	White tablets	White tablets
Assay (%)	95.8	103.2	99.4
Dissolution (%):			
3 hr	24	10	10
6 hr	41	22	21
12 hr	62	50	46
24 hr	92	90	84
200 mg tablet, Formulation K, 25 °C/60% RH			
Appearance	White tablets	White tablets	White tablets
Assay (%)	95.9	100.9	100.3
Dissolution (%):			
3 hr	34	15	13
6 hr	53	31	30
12 hr	76	69	68
24 hr	104	101	96

Test	Time point		
	Initial	1 month	2 months
200 mg tablet, Formulation K, 40 °C/75% RH			
Appearance	White tablets	White tablets	White tablets
Assay (%)	95.9	99.6	100.0
Dissolution (%):			
3 hr	34	10	10
6 hr	53	26	28
12 hr	76	60	61
24 hr	104	90	90

3.5. A controlled release dosage form of diazoxide or a derivative thereof using an osmotically controlled release system.

[00612] Diazoxide salt or a derivative thereof is formulated as an osmotically regulated release system. In general, two components, and an expandable hydrogel that drives release of the active drug is assembled with diazoxide salt or a derivative thereof into a semipermeable bilaminate shell. Upon assembly a hole is drilled in the shell to facilitate release of active upon hydration of the hydrogel.

[00613] A dosage form adapted, designed and shaped as an osmotic delivery system is manufactured as follows: first, a diazoxide salt or a derivative thereof composition is provided by blending together into a homogeneous blend of polyethylene oxide, diazoxide salt or a derivative thereof and hydroxypropyl methylcellulose. Then, a volume of denatured anhydrous ethanol weighing 70% of the dry mass is added slowly with continuous mixing over 5 minutes. The freshly prepared wet granulation is screened through a 20 mesh screen through a 20 mesh screen, dried at room temperature for 16 hours, and again screened through a 20 mesh screen. Finally, the screened granulation is mixed with 0.5% weight of magnesium stearate for 5 minutes.

[00614] A hydrogel composition is prepared as follows: first, 69% weight of polyethylene oxide weight, 25% weight of sodium chloride and 1% weight ferric oxide

are separately screened through a 40 mesh screen. Then, all the screened ingredients are mixed with 5% weight of hydroxypropyl methylcellulose to produce a homogeneous blend. Next, a volume of denatured anhydrous alcohol equal to 50% of the dry mass is added slowly to the blend with continuous mixing for 5 minutes. The freshly prepared wet granulation is passed through a 20 mesh screen, allowed to dry at room temperature for 16 hours, and again passed through a 20 mesh screen. The screened granulation is mixed with 0.5% weight of magnesium stearate for 5 minutes (see U.S. Patent No. 6,361,795 by Kuczynski, et al.).

[00615] The diazoxide salt composition, or a derivative thereof, and the hydrogel composition, are compressed into bilaminate tablets. First the diazoxide salt or a derivative thereof composition is added and tamped. The hydrogel composition is then added and the laminae are pressed under a pressure head of 2 tons into a contacting laminated arrangement.

[00616] The bilaminate arrangements are coated with a semipermeable wall (i.e. thin film). The wall forming composition comprises 93% cellulose acetate having a 39.8% acetyl content, and 7% polyethylene glycol. The wall forming composition is sprayed onto and around the bilaminate.

[00617] Finally, an exit passageway can be drilled through the semipermeable wall to connect the diazoxide salt or a derivative thereof drug lamina with the exterior of the dosage system. Residual solvent is removed by drying at 50° C and 50% humidity. The osmotic systems are dried at 50° C to remove excess moisture (see U.S. Patent No. 6,361,795 by Kuczynski, et al.).

4. Preparation of Diazoxide Salts

4.1. Preparation of the Sodium Salt

[00618] The sodium salt of diazoxide was prepared by dissolving 300 mg of diazoxide in approximately 45 mL methyl ethyl ketone (MEK). The diazoxide/MEK solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added 1.3 mL of 1M NaOH (1 molar equivalent). The combined solutions were heated at 75°C for

approximately 30 minutes and allowed to cool to room temperature. The mixture was concentrated under reduced pressure, and dried in vacuo at 55°C and 30 in. Hg. Elemental analysis: Calculated, 38.03% C, 2.39% H, 11.09% N and 9.1% Na; Found, 38.40% C, 2.25% H, 10.83% N and 7.4% Na.

[00619] A sodium salt of diazoxide was also prepared by dissolving 300 mg of diazoxide in approximately 45 mL acetonitrile. The diazoxide/acetonitrile solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added 1.3 mL of 1M NaOH (approximately 1 molar equivalent). The combined solutions were heated at 75°C for approximately 30 minutes and allowed to cool to room temperature. The mixture was concentrated under reduced pressure, and dried in vacuo at 55°C and 30 in. Hg.

4.2. Preparation of the Potassium Salt

[00620] The potassium salt of diazoxide was prepared by dissolving 300 mg of diazoxide in approximately 45 mL methyl ethyl ketone (MEK). The diazoxide/MEK solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added approximately 1.3 mL of 1 M KOH (1 molar equivalent) and the solution was returned to the orbital shaker, heated at 75°C for approximately 30 minutes and allowed to cool to room temperature. The solvent was removed under reduced pressure and the solid was dried in vacuo at 55°C and 30 in. Hg. Elemental analysis: Calculated, 33.59% C, 2.81% H, 9.77% N and 13.63% K; Found, 34.71% C, 2.62% H, 9.60% N and 10.60% K.

[00621] The potassium salt of diazoxide was also prepared by dissolving 300 mg of diazoxide in approximately 45 mL tetrahydrofuran (THF). The diazoxide/THF solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added approximately 1.3 mL of 1 M KOH (1 molar equivalent) and the resulting solution was returned to the orbital shaker, heated at 75°C for approximately 30 minutes and allowed to cool to room temperature. THF was removed under reduced pressure and the solid was dried in vacuo at 55°C and 30 in. Hg.

APOTEX_EX1009_PGR2026-00034_Page 152

4.3. Preparation of Choline Salt

4.3.1. Preparation of the Choline Salt: proof of concept

[00622] The choline salt of diazoxide was prepared by dissolving 300 mg of diazoxide in approximately 45 mL methyl ethyl ketone (MEK). The diazoxide/MEK solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added approximately 315 mg of 50 wt. % of choline hydroxide (1 molar equivalent) and the solution was returned to the orbital shaker and stirred at 75°C for approximately 30 minutes. The solvent was removed under reduced pressure, and the solid was dried in vacuo at 55°C and 30 in. Hg. Elemental analysis: Calculated, 46.77% C, 5.86% H, and 12.00% N; Found, 46.25% C, 6.04% H, and 12.59% N.

4.3.2. Process of preparation of the Choline Salt

[00623] An investigation of the minimum solvent volumes required to optimize the formation of the diazoxide choline salt was performed in MeCN, THF, MEK, and 2-MeTHF on small scale in the presence and absence of MTBE. Analyses by 1H NMR and XRPD of all reactions were consistent with the assigned structure and Form B of diazoxide choline salt.

4.3.2.1. Solvent Efficiency Study with Single Solvent System.

[00624] Investigation of single solvent system synthesis of diazoxide choline salt using solvents THF, MEK, and 2-MeTHF were conducted, results of which are shown in Table 8. These results suggest that precipitation can be achieved with a single solvent system in THF or 2-MeTHF. The best results, (i.e., Table 8 entries 14-17) obtained with 2-MeTHF. However, it was observed that in 2-MeTHF at 3-volumes and above, complete dissolution was not achieved after addition of choline hydroxide.

Table 8. Solvent Efficiency of a Single Solvent System

Entry	Solvent	Solvent volume	Yield (%)	Polymorphic Form

Entry	Solvent	Solvent volume	Yield (%)	Polymorphic Form
1	THF	1.0	7	B
2	THF	2.0	n/a	n/a
3	THF	3.0	20	B
4	THF	4.0	28	B
5	THF	5.0	32	B
6	THF	6.0	42	B
7	THF	7.0	53	B
8	THF	8.0	50	B
9	MEK	4.0	n/a	n/a
10	2-MeTHF	1.0	n/a	n/a
11	2-MeTHF	2.0	48	B
12	2-MeTHF	3.0	41	B
13	2-MeTHF	4.0	48	B
14	2-MeTHF	5.0	71	B
15	2-MeTHF	6.0	71	B
16	2-MeTHF	7.0	72	B
17	2-MeTHF	8.0	67	B

n/a: Samples were either seen to form a gum, or no precipitate was observed.

4.3.2.2. Solvent Efficiency Study with Binary Solvent Systems.

[00625] The addition of a second solvent at 1-20 volumes (i.e., 1-8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) to the solvent volumes of Table 8 was envisaged to optimize diazoxide salt production yields. Results of investigation of methods to enhance precipitation and increase yield employing binary solvent system synthesis of diazoxide choline salt using solvents THF, MeCN, MEK, and 2-MeTHF and co-solvent MTBE are shown in Table 9. Optimum conditions for the preparation of the diazoxide choline salt obtained with 1-3 volumes of THF (see Table 8, entries 1-3), with 3 volumes being the

ratio of choice to eliminate excessive drag during stirring of slurry in large scale production.

Table 9. Solvent Efficiency of a Binary Solvent System

Entry	Solvent	Solvent Volume	Co-Solvent (12 Vol)	Yield (%)	Polymorphic Form
1	THF	1.0	MTBE	96	B
2	THF	2.0	MTBE	96	B
3	THF	3.0	MTBE	91	B
4	THF	4.0	MTBE	90	B
5	THF	5.0	MTBE	84	B
6	THF	6.0	MTBE	90	B
7	THF	7.0	MTBE	94	B
8	THF	8.0	MTBE	94	B
9	MeCN	1.0	MTBE	92	B
10	MeCN	2.0	MTBE	90	B
11	MeCN	3.0	MTBE	87	B
12	MeCN	4.0	MTBE	79	B
13	MeCN	5.0	MTBE	79	B
14	MeCN	6.0	MTBE	75	B
15	MeCN	7.0	MTBE	70	B
16	MeCN	8.0	MTBE	74	B
17	MEK	4.0	MTBE	83	B
18	2-MeTHF	1.0	MTBE	97	B
19	2-MeTHF	2.0	MTBE	94	B
20	2-MeTHF	3.0	MTBE	94	B
21	2-MeTHF	4.0	MTBE	95	B
22	2-MeTHF	5.0	MTBE	97	B

APOTEX_EX1009_PGR2026-00034_Page 155

Entry	Solvent	Solvent Volume	Co-Solvent (12 Vol)	Yield (%)	Polymorphic Form
23	2-MeTHF	6.0	MTBE	94	B
24	2-MeTHF	7.0	MTBE	97	B
25	2-MeTHF	8.0	MTBE	>99	B

4.3.2.3. Co-Solvent Efficiency and Optimization with MTBE.

[00626] Results of optimization of co-solvent (MTBE) volume in the presence of 3 volumes of solvent are shown in Table 10.

Table 10. Co-Solvent Efficiency and Optimization with MTBE

Entry	Solvent	MTBE Volume	Yield (%)	Form
1	MeCN	8.0	76	B
2	MeCN	14.0	85	B
3	THF	6.0	93	B
4	THF	8.0	93	B
5	THF	14.0	98	B

[00627] Optimized conditions for the production of the choline salt of diazoxide in a binary solvent system obtained with 3:14 (i.e., 1:4.7) THF/MTBE (v/v).

4.3.2.4. Optimization of Cooling Profile.

[00628] Optimization for the controlled precipitation of diazoxide choline salt was conducted by varying the cooling temperatures and hold times. Reactions were carried out in MeCN, THF, and 2-MeTHF (3 vol) with MTBE (14 vol). The results are presented in Table 11. These results suggest that optimum crystal growth was achieved with cooling to 0–5 °C for eight hours in THF and 2-MeTHF (Entries 5–6) when compared to those in MeCN (Entries 1, 2, and 4). These results however did not indicate enhanced precipitation when compared to previous studies carried out in THF and 2-MeTHF when the slurries were allowed to stir for two hours. In addition no notable improvements were

observed for the additional cooling to –15 °C or with the filtration at ambient temperature.

Table 11. Cooling Profile Optimization for Controlled Precipitation

Entry	Solvent	Cooling Temp. (°C)	Hold Time (hr)	Yield (%)	Form
1	MeCN	0–5	2	89	B
2	MeCN	–10 → –15	2	85	B
3	THF	RT	2	80	B
4	MeCN	RT	2	85	B
5	THF	0–5	8	94	B
6	2-MeTHF	0–5	8	95	B

4.3.2.5. Optimization of Rate of Co-solvent addition.

[00629] The effect of rate and hold time of co-solvent addition on the precipitation of diazoxide choline salt was examined. The reactions were carried out on 500 mg scale in three volumes of primary solvent and 14 volumes of MTBE (THF and 2-MeTHF) or 1:3 volume ratio (MeCN). Addition of the co-solvent was made dropwise in one portion for Entry 1, and for Entries 2 and 3 the co-solvent was added at a rate of 1 mL/20 minutes for a total hold time of 140 minutes for the addition of 7 mL. The results, presented in Table 12, indicate that optimum precipitation of diazoxide choline salt was achieved by the addition of MTBE with 20 minute hold times in between additions in THF or 2-MeTHF (entries 2-3).

Table 12. Optimization of Rate of Co-solvent addition

Entry	Solvent	Hold Time (min)	Addition Rate (mL/min)	Yield (%)	Form
1	MeCN	60	n/a	87	B
2	THF	140	1.0/20	96	B
3	2-MeTHF	140	1.0/20	96	B

4.3.2.6. Thermal Stability Study.

[00630] The stability of the isolated diazoxide choline salt was investigated to determine optimal drying conditions to minimize residual solvents without degradation. Samples of the diazoxide choline salt prepared in THF and MTBE (Entries 1–4) were dried under vacuum at 40 °C for various durations on small scale (100 mg). The study was then carried out on 5 g scale (Entry 5) at 30 °C for eight hours under vacuum to show reproducibility on large scale. The results are presented in Table 13. Analyses of the samples of Table 13 by ^{1}H NMR, XRPD, and HPLC indicated that elevated temperatures and prolonged drying times did not degrade the compound or affect the form of the compound.

Table 13. Thermal Stability Study

Entry	Drying Temp (C)	Time (h)	OVI (THF/MTBE) (ppm)	Polymorphic Form
1	RT	12	376/248	B
2	40	20	317/160	B
3	40	28	276/127	B
4	40	42	268/128	B
5	30	8	241/509	B

4.3.2.6. Demonstration of 50-g Scale synthesis of Diazoxide Choline Salt in THF.

[00631] The preparation of the diazoxide choline salt was carried out in a binary-solvent system of THF and MTBE with a ratio of 1:4.7 (solvent/co-solvent) volumes and cooling to 0–5 °C for two hours with stirring. A demonstration run for the large scale production utilizing the modified procedure was carried out on 50 g-scale. Diazoxide (50 g) as a hot (62 °C) suspension in THF (140 mL) was treated with choline hydroxide (45% solution in MeOH, 1.0 equiv) added (2 mL/min) over 30 minutes. The resulting solution was stirred for 30 minutes, followed by cooling to 52 °C for the addition of MTBE (14

vol) over 45 minutes. On addition of two volumes of co-solvent precipitation was observed. The resultant slurry was then allowed to cool naturally to ambient temperature followed by further cooling to 0–5 °C with an ice/water bath and an additional 2 hr stirring. The precipitate was isolated by vacuum filtration, and the filter cake rinsed with ice cold MTBE (~50 mL) and dried under vacuum at room temperature for 12 hours. OVI analysis indicated that THF levels were above the recommended ICH guidelines (799 ppm) and the material was returned to the oven at 30 °C for eight hours to give diazoxide choline salt [70.87 g, 97% yield, 97% purity AUC] as a white crystalline solid. Analyses by 1H NMR, XRPD, and HPLC were consistent with the assigned structure of Form B while maintaining a high purity with good yield.

4.3.2.7. Demonstration of 50-g Scale synthesis of Diazoxide Choline Salt in 2-MeTHF.

[00632] A 50-g level synthesis was duplicated in 2-MeTHF/MTBE as an alternative to THF/MTBE for the large scale production of the diazoxide choline salt described above. No issues or concerns arose during the reaction other than complete dissolution was not achieved after addition of the choline hydroxide. A white precipitate formed after addition of the co-solvent, which was isolated via vacuum filtration and dried under vacuum at 30 °C for eight hours to give diazoxide choline salt [71.51 g, 97% yield] as a white crystalline solid. Analyses by 1H, XRPD, and HPLC were consistent with the assigned structure and Form B. OVI analysis showed 2-MeTHF and MTBE levels of 125 and 191 ppm, respectively, which were below the ICH guidelines.

4.3.2.8. 250-g Scale synthesis of Diazoxide Choline Salt in THF.

[00633] Larger scale preparation of the diazoxide choline salt was carried out in a binary-solvent system of THF and MTBE with a ratio of 1:4.7 (v/v) volumes. Diazoxide as a hot (62 °C) suspension in THF (745 mL) was treated with choline hydroxide as a 45% solution in MeOH (1.0 equiv) added over 30 minutes. The resulting solution was stirred for 30 minutes, followed by cooling to 52 °C for the addition of MTBE (14 vol) over 45 minutes. On addition of two volumes of co-solvent, precipitation was observed. The resultant slurry was then allowed to cool naturally to ambient temperature followed

by cooling to 0–5 °C with an ice/water bath. The precipitate was isolated by vacuum filtration, and the filter cake rinsed with ice cold MTBE (approximately 250 mL) and dried under vacuum at 30 °C for 38 hours to give diazoxide choline salt (350.28 g, 97.7% yield) as a white crystalline solid. Analyses by 1H NMR, XRPD, and HPLC were consistent with the assigned structure of Form B while maintaining a high purity with good yield.

4.3.2.9. 2-kg Scale Synthesis of Diazoxide Choline Salt in THF.

[00634] A 12-L reaction flash was charged with 2.0 kg diazoxide and 5.0-L THF with stirring and heating to 55 °C. Choline hydroxide (45% solution in methanol, 2.32 L) was added dropwise to this reaction mixture over about 2.5 hr with stirring. The temperature was maintained at 60 ± 5 °C. After addition of choline hydroxide, stirring was continued for about 30 min. The reaction mixture was clarified by in-line 10 micron filtration upon transfer to a 22-L reaction flask pre-charged with 2-L pre-filtered THF, into which was added 10-L pre-filtered MTBE dropwise. This reaction mixture was transferred to another flask which was then charged with an additional 30-L pre-filtered MTBE dropwise, with adjustment of temperature to < 5 °C and stirring for about 2 hr. Diazoxide choline salt was recovered by vacuum filtration to afford 2.724 kg (94%) diazoxide choline salt (99.8%, HPLC purity), confirmed by 1H NMR, IR, and UV/Visible analysis.

4.4. Preparation of the Hexamethyl Hexamethylene Diammonium Hydroxide Salt

[00635] The hexamethyl hexamethylene ammonium salt of diazoxide was prepared by dissolving 50 mg of diazoxide in approximately 7.5 mL methyl ethyl ketone (MEK). The diazoxide/MEK solution was heated at 75°C on an orbital shaker to ensure dissolution. To the solution was added approximately 2.17mL of 0.1M hexamethyl hexamethylene ammonium hydroxide solution (1 molar equivalent) and the solution was stirred at 75°C for an additional 10 minutes and then cooled to room temperature at the rate of 30°C/h. The solvent was removed under reduced pressure, and the solid was dried in vacuo at 55°C and 30 in. Hg.

4.5. Failure to Obtain Salts of Diazoxide and derivatives

4.5.1. Failure to Obtain Salts from Alkali Metal Hydroxides

[00636] U.S. Patent Number 2,986,573 ("the '573 patent") describes the synthesis of diazoxide metal salts in aqueous or non-aqueous solutions in the presence of an alkali metal alkoxide. According to the '573 Patent, diazoxide can be dissolved in an alkali metal solution, and the salt obtained upon evaporation. Also described is a method for forming salts from non-aqueous media wherein diazoxide and sodium methoxide are dissolved in anhydrous methanol and the solvent is evaporated to obtain the sodium salt of diazoxide as a white solid.

[00637] Attempts were made to prepare a diazoxide salt from alkali metal hydroxides by the method described in the '573 patent. Salt preparation was carried out in aqueous media by dissolving diazoxide in a basic solution 1M NaOH, followed by evaporation of the solvent. A solid was obtained and analyzed by XRPD (X-Ray Powder Diffraction) and NMR. However, this analysis confirmed that the solid obtained was the diazoxide starting material and not a salt.

[00638] Salt preparation was carried out in non-aqueous media by dissolving diazoxide in anhydrous methanol in the presence of either sodium methoxide or potassium methoxide and stirring the mixture at 60°C for 15 minutes. The mixture was then cooled to room temperature while stirred. After approximately two hours, a solid was recovered, isolated by filtration, and dried in vacuo. Analysis by XRPD confirmed that the solid obtained was the diazoxide starting material and not a salt.

4.5.2. Preparation of Salts in Methanol or Ethanol According to the '573 Patent

[00639] The preparation of diazoxide salts in methanol and ethanol was attempted using 22 different counter-ions according to the methods described by the '573 Patent. For example, 20 mg of diazoxide was dissolved in 5 mL of ethanol and stirred and heated to ensure dissolution of the diazoxide. To the stirred solution was added approximately 1

molar equivalents of sodium methoxide. The solution was stirred for approximately 10-15 minutes at 60°C, and cooled to room temperature for approximately 2 hours. The resulting solid precipitate was concentrated under a nitrogen stream, and collected by filtration. The product was dried in vacuo and analyzed by XRPD. As shown in Figure-15, XRPD of the solids collected from the sodium methoxide experiment, (as well as the solids collected from the potassium methoxide experiment, which was run under similar conditions), revealed that the solid was the diazoxide starting material and that no sodium or potassium salt was prepared. In Figure 15, (a) is the XRPD pattern of free form diazoxide, (b) is the XRPD pattern of the product of potassium methoxide in methanol, and (c) is the XRPD pattern of the product of sodium methoxide in methanol.

[00640] Although not wishing to be bound by any theory, it is believed that a possible explanation for the failure to prepare diazoxide salts by the methods of the '573 Patent (i.e., in the presence of alcohols, e.g., methanol or ethanol), is that the alcohol may have an effect on the stability of the alkali salts of diazoxide. This was supported by UV spectroscopy analysis of alkali salts of diazoxide (sodium and potassium). Both the sodium and potassium salts of diazoxide were synthesized by reacting diazoxide with NaOH or KOH in MEK. Elemental analysis, NMR and XRPD confirmed that the salts were made.

[00641] Upon dissolution of the sodium or the potassium salt in acetonitrile, the UV spectrum shows a shift into the red region of the spectrum for the λmax from approximately 268 nm for the free form of diazoxide to approximately 298 nm for both the potassium and sodium salt (see Figure 1). Similarly, these salts can be stabilized in aqueous solutions by elevating the pH to above 9.0. A similar shift in the absorption maximum at pH 9.0 is measured using UV spectroscopy. Subsequent adjustment of the pH from greater than 9.0 to less than 6.2 results in the hydrolysis of the salt as measured by the recovery of the UV absorption pattern of the diazoxide free base (see Figure 2). In contrast, when the sodium or the potassium salt is dissolved in methanol, the UV-Vis spectrum of the salt was identical to that of the diazoxide starting material, (see Figure 3).

[00642] These results demonstrate that using methanol as the solvent is incompatible with the synthesis of alkali salts of diazoxide. In addition, the results show that isolation of an alkali metal salt of diazoxide (or any other salt) in the presence of an alcohol, such

as methanol, may not be possible.

4.5.3. Failure to Obtain Salts from Acidic Counter Ions

[00643] Salt formation with diazoxide was also attempted using acidic counter-ions, such as, for example, hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, benzoic acid, undecylenic acid, salicylic acid and quinic acid. However, no salt formation was observed for any acid in any solvent.

[00644] For example, 100 mg of diazoxide was dissolved in 50 mL of acetone and heated to 35°C. To a stirred solution was added approximately 4.65 molar equivalents of HCl. The reaction mixture was allowed to cool to room temperature for approximately 3 hours, with no precipitation observed. Solvent was removed in vacuo. The resulting solid was analyzed by XRPD, and the observed XRPD pattern was consistent with the free form diazoxide starting material. In all cases, the attempted synthesis of diazoxide salts from acids was unsuccessful in all solvents attempted.

4.6. Preparation of Salts of Compounds of Formulae V-VIII

[00645] The chloride salt of 3-amino-4-methyl-1,2,4-benzothiadiazine-1,1-dioxide is prepared dissolving approximately 300 mg (1.4 mmol) in 45 mL acetonitrile. The mixture is heated to approximately 75°C and stirred for 30 min. To the stirred solution, approximately 1 molar equivalent of HCl is added dropwise, and stirred for approximately 30 min at 75°C. The mixture is cooled to room temperature and the solvent is removed under reduced pressure, affording the chloride salt as a solid.

[00646] The sodium salt of 3-amino-4-methyl-1,2,4-benzothiadiazine-1,1-dioxide is prepared dissolving approximately 300 mg (1.4 mmol) in 45 mL acetonitrile. The mixture is heated to approximately 75°C and stirred for 30 min. To the stirred solution, approximately 1 molar equivalent of NaOH is added dropwise, and stirred for approximately 30 min at 75°C. The mixture is cooled to room temperature and the solvent is removed under reduced pressure, affording the sodium salt as a solid.

5. Characterization of Prepared Diazoxide Salts

[00647] Synthesis of the desired salts was confirmed by X-Ray Powder Diffraction (XRPD), UV-Vis spectroscopy, and NMR. All spectra were compared with the spectra of the free form diazoxide (i.e., not a salt). Differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), FTIR (Fourier transform infrared spectroscopy), NMR, UV-vis spectroscopy and moisture sorption analysis were also performed.

5.1. Experimental Procedures

[00648] DSC analysis were conducted with a Mettler 822 DSC, by measuring the amount of energy released by a sample, as the sample was heated from 30°C to between 300-500°C at a rate of 10°C/min. Typical applications of DSC analysis include determination of melting point temperature and the heat of melting; measurement of the glass transition temperature; curing and crystallization studies; and identification of phase transformations.

[00649] TGA measurements were conducted with a Mettler 851 SDTA/TGA, by measuring weight loss as a function of increasing temperature, as the samples were heated from 30°C to 230°C at a rate of 10°C/min. The TGA can be used to analyze deabsorption and decomposition behavior, characterize oxidation behavior, set burnout or conditioning parameters (temperature/ramp rate/time), and determine chemical composition.

[00650] XRPD samples were analyzed with a Shimadzu XRD-6000 system, using a Cu Kα, 40 kV, 40 mA X-ray tube. The divergence and scatter slits were 1.00 deg, and the receiving slit was 0.30 mm. Samples were continuously scanned at a range of 3.0-45.0 deg, with a step size of 0.04 deg., at a scan rate of 2 deg/min.

[00651] Fourier Transform Infrared Spectroscopy was measured with a Thermo-Nicolet Avatar 370 with a Smart Endurance Attenuated Total Reflection (ATR) attachment. Compressed samples were analyzed, with corrections for background noise being made. Using the IR spectrum, chemical bonds and the molecular structure of organic compounds can be identified. Attenuated total reflectance (ATR) allows for the analysis of thin films, organic and inorganic, in areas as small as 10-15 microns.

[00652] Nuclear Magnetic Resonance (NMR) was performed with a 400 MHz Bruker Avance with a 4mm CP/MAS H-X probe. Acquisition of 1H NMR spectra were performed by taking between 5-10 mg of the sample, dissolved in approximately 0.78 mL of DMSO-d6. Spectra were acquired with either 16 or 32 scans, using a pulse delay of 1.0 sec, with a 10 μsec (30°) pulse width.

[00653] UV spectroscopy was performed with a Perkin-Elmer Lambda 25 spectrometer. Samples were dissolved in acetonitrile, water and a buffer system having a pH between 5.6 and 10. Spectra were acquired between 340 and 190 nm, using a 1 cm path length with background correction.

[00654] Moisture Sorption Analysis was performed with a Hiden IGAsorp Moisture Sorption Instrument. Samples were first dried at 0% relative humidity at 25°C until an equilibrium weight was reached, or for a maximum of 4 hours. Samples were then subjected to an isothermal (25°C) scan from 10 – 90% relative humidity in steps of 10%. The samples were allowed to equilibrate to an asymptotic weight at each point for a maximum of 4 hours. Following absorption, a desorption scan from 85% relative humidity (at 25°C) was run in steps of -10%, again allowing a maximum of 4 hours for the samples to equilibrate. The resulting samples after desorption were dried at 80°C for two hours and analyzed by XRPD.

5.2. Free Form Diazoxide Characterization

[00655] The free form of diazoxide was characterized by XRPD, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), moisture sorption, 1H NMR, FTIR and UV-vis spectroscopy to provide a baseline for comparison with the salts. Free form diazoxide is highly crystalline, as shown by the XRPD pattern. (See Figure 4(a)). The DSC shows a large endothermic event at 330°C, and TGA shows that the free form of diazoxide is anhydrous, where diazoxide shows no weight loss below 200°C, and a weight loss of only 0.2% below 230°C. Moisture absorption of the free form diazoxide shows the material to be non-hygroscopic. Absorption of water by diazoxide was tested at between 0 – 90% relative humidity (RH) at 25°C, showing absorption of approximately 0.04 wt% at 60% RH and 0.20 wt% at 90% RH. The molecule does not form a stable hydrate, as shown by the lack of hysteresis during desorption. Additionally, the XRPD

pattern for the diazoxide before and after absorption of water indicate the same crystalline form.

[00656] UV-vis spectroscopy measurements taken of the free form diazoxide in neutral aqueous solution show a λmax at approximately 268 nm. In acetonitrile, the λmax was 264 nm, demonstrating a small solvatochromic shift. As shown in Figure 2, as pH increased the λmax also increased, from approximately 265 nm to approximately 280 nm, due to a change in the electronics of the molecule.

[00657] Studies were also conducted to evaluate the likelihood for conversion and degradation under thermal stress. Samples were heated in a closed environment, protected from light, at 60°C for approximately 14 days. The diazoxide showed no conversion or degradation at 7 days or 14 days. Diazoxide samples were found to be consistent with the starting material with respect to XRPD and DSC.

[00658] Slurry studies to determine propensity of inter-conversion of the solid form were conducted on the free form diazoxide at room temperature, in the absence of light, using water, isopropyl alcohol, dichloromethane and toluene. Approximately 20 mg of the free form diazoxide was stirred for 14 days. Analysis by XRPD, DSC and HPLC were consistent with the starting material, indicating that the free form of diazoxide did not convert to alternate crystal forms.

5.3. Characterization of Sodium Diazoxide Salt

[00659] The XRPD pattern of the sodium salt of diazoxide was analyzed, showing the material to be crystalline. (See Figure 4(d)). The DSC analysis revealed a major exothermic event at 448°C. Small transitions below 400°C are likely due to sample imperfections. TGA analysis showed weight loss of 0.2% and 0.03% below 120°C, which may be the result of bound solvent. Moisture absorption performed from 0 – 90% relative humidity at 25°C showed the material to be hygroscopic as the sample deliquesced at 90% relative humidity. The sample absorbed 1.2 wt% of water at 60% RH, and 6.6 wt% water at 80% RH. Hysteresis was observed upon desorption at 65 and 55% RH, indicating possible hydrate formation. (Possible hydrate formation was noted, although the amount of water absorbed was less than 0.5 mole). 1H NMR showed a

APOTEX_EX1009_PGR2026-00034_Page 166

chemical shift in the aromatic and methyl resonances of the sodium salt, as expected due to changes to the aromatic system. See Figure 5 showing NMR spectrum for the free form of diazoxide (a)and the sodium salt of diazoxide (c). FTIR showed expected changes for the sodium salt.

[00660] Elemental analysis of the salt indicated that the salt was formed in a ratio of approximately 1:1, with the percentage of sodium being slightly low (approximately 3.4%). This deficiency may be due to matrix effects, as NMR indicated the sample had a relatively high purity.

[00661] UV-vis measurements in neutral aqueous solution show a λmax of approximately 271 nm. (See Figure 1). This value is slightly higher than free form diazoxide (265 nm). In acetonitrile, the λmax of the sodium salt exhibits a solvatochromic shift to approximately 296 nm. (See Figure 3). An increase in the pH of the solution is expected to produce a bathochromic shift from approximately 265 nm to approximately 280 nm.

[00662] Solubility measurements performed at pH 2, 7, and 12 in 10 mM phosphate buffer at room temperature showed solubility of the sodium salt of diazoxide to be 13.0 mg/mL, 18.1 mg/mL and 48.6 mg/mL, respectively.

[00663] Form conversion and degradation under thermal stress were conducted as described for the free form diazoxide salt and showed the salt had little propensity to change form or degrade over a period of 14 days. Similarly, slurry studies were conducted as described for the free form diazoxide in n-heptane, dichloromethane and toluene showed no propensity of inter-conversion. See Figure 6, wherein (a) is the XRPD pattern for the sodium salt of diazoxide, (b) is the XRPD pattern for the sodium salt after the slurry study, and (c) the XRPD of the free form of diazoxide.

5.4. Characterization of Potassium Diazoxide Salt

[00664] The XRPD pattern for the potassium salt of diazoxide was analyzed, showing the material to be crystalline. (See Figure 4(b)). The DSC analysis revealed two major exothermic events at 128 and 354°C. (See Figure 7). Small endotherms are likely due to

sample impurities or the presence of solvent. TGA analysis showed weight loss of 7.7% below 220°C, which may be the result of moisture sorption. (See Figure 8). Theoretical weight loss for a monohydrate of the diazoxide salt is 6.6%. Moisture absorption performed from 0 – 90% relative humidity at 25°C showed the material to be hygroscopic as the sample deliquesced at 90% relative humidity, showing 38.3 wt% water. The sample absorbed 5.4 wt% of water at 60% RH. Hysteresis was observed upon desorption, however the material was determined to be a hemihydrate from 0 – 30% RH and a monohydrate from 35 – 75% RH. XRPD following the desorption analysis indicated that the sample had changed to an alternate crystalline form. 1H NMR showed a chemical shift in the aromatic and methyl resonances of the sodium salt, as expected due to changes to the aromatic system. (See Figure 5 showing spectrum of the free form diazoxide (a) and the Potassium salt of diazoxide (b)). FTIR showed expected changes for the potassium salt.

[00665] Elemental analysis of the potassium salt indicated that the salt was formed in a ratio of approximately 1:1, with the percentage of potassium being slightly low (approximately 1.6%). This deficiency may be due to matrix effects, as NMR indicated the sample had a relatively high purity.

[00666] UV-vis measurements of the potassium diazoxide salt in neutral aqueous solution show a λmax of approximately 265 nm, which is equivalent to the diazoxide free form λmax. (See Figure 1. In acetonitrile, the λmax of the potassium salt exhibits a solvatochromic shift to approximately 296 nm. (See Figure 3). The potassium salt was used in a pH dependency study and showed that increasing the pH of the solution resulted in a bathochromic shift of the λmax from approximately 265 nm to approximately 280 nm.

[00667] Solubility measurements performed at pH 2, 7, and 12 in 10 mM phosphate buffer at room temperature showed solubility of the sodium salt of diazoxide to be 9.9 mg/mL, 14.4 mg/mL and 43.0 mg/mL, respectively. The potassium salt displayed greater solubility than the free form diazoxide, and demonstrated similar solubility to the sodium diazoxide salt. The XRPD pattern of solids obtained after the solubility analysis indicated that the potassium salt had changed back to the free form diazoxide material.

APOTEX_EX1009_PGR2026-00034_Page 168

[00668] Propensity for form conversion and degradation under thermal stress were conducted as described for the free form diazoxide salt. The XRPD pattern of the sample after 7 and 14 days showed unique peaks, as compared with the potassium salt starting material. Analysis by DSC after 14 days also showed unique peaks as well. Using a gradient area percent assay, HPLC did not show any significant degradation of the potassium salt.

[00669] Slurry studies were conducted as described for the free form diazoxide in n-heptane, dichloromethane and toluene showed no propensity of inter-conversion. XRPD analysis of samples after 7 and 14 days showed unique peaks similar to those observed with the thermal stress study. See Figure 9, wherein (a) is the XRPD pattern of the potassium salt of diazoxide, (b) is the XRPD pattern of the potassium salt of diazoxide after the slurry study in toluene, and (c) the XRPD pattern of the potassium salt after 14 days of the slurry study in toluene. Analysis by DSC after 14 days also showed unique peaks as compared with the starting material. HPLC using a gradient area percent assay did not show any significant degradation after the study.

5.5. Characterization of Choline Diazoxide Salt

[00670] The XRPD pattern of the choline salt of diazoxide was analyzed, showing the material to be crystalline. (See Figure 10 (b)). The DSC analysis revealed a major exothermic events at 167°C. (See Figure 11). A smaller endothermic event was seen at 119°C and is likely due to sample impurities or the presence of residual solvent. TGA analysis showed weight loss of 0.8% between 100 and 140°C, which may be the result of residual solvents. (See Figure 12). Moisture absorption performed from 0 – 90% relative humidity at 25°C showed the material to be hygroscopic as the sample absorbed over 28% at 80% relative humidity, and deliquesced at 90% RH. Hysteresis was not observed upon desorption, indicating the choline salt does not form a stable hydrate. The XRPD pattern following the desorption analysis indicated that the sample had changed to an alternate crystalline form during the hydration and subsequent desorption. (See Figure 13 (c)) 1H NMR was consistent with a 1:1 molar ratio of diazoxide to counter-ion, and had the expected differences in the chemical shift of the aromatic and methyl resonances, as expected due to the change in the local environment of the aromatic system due to the

presence of the choline counter-ion. (See Figure 14 (b)). FTIR showed expected changes for the choline salt, similar to those seen with the sodium and potassium salts.

[00671] Elemental analysis of the choline salt indicated that the salt was formed in a ratio of approximately 1:1. This is consistent with the 1H NMR.

[00672] UV-vis measurements of the choline diazoxide salt in neutral aqueous solution show a λmax of approximately 268 nm, which is close to the λmax for the diazoxide free form of 265 nm. In acetonitrile, the λmax of the choline salt exhibits a solvatochromic shift to approximately 296 nm, which is consistent with the sodium and potassium diazoxide salts. The potassium salt was used in a pH dependency study and showed that increasing the pH of the solution resulted in a bathochromic shift of the λmax from approximately 265 nm to approximately 280 nm.

[00673] Solubility measurements performed at pH 2, 7, and 12 in 10 mM phosphate buffer at room temperature showed solubility of the sodium salt of diazoxide to be 28.2 mg/mL, 41.5 mg/mL and greater than 293 mg/mL, respectively. The choline salt displayed greater solubility than the free form diazoxide after being allowed to equilibrate for 12 hours. The XRPD pattern of the solids obtained after the solubility analysis (at pH 2 and 7 only) indicated that the choline salt had changed back to the free form diazoxide material.

[00674] Propensity of the choline salt for form conversion and degradation under thermal stress were conducted as described for the free form diazoxide salt. XRPD analysis of the sample after 7 and 14 days showed an XRPD pattern consistent with the starting material, as well as the presence of additional unique peaks. (More unique peaks were present after 14 days than after 7 days). Analysis by DSC after 14 days did not show any significant difference, with a small endotherm at 117°C and a large endotherm at 168°C. (Initial DSC showed endotherms at 119°C and a large endotherm at 167°C). Using a gradient area percent assay, HPLC did not show any significant degradation of the choline salt.

[00675] Slurry studies were conducted as described for the free form diazoxide in n-heptane, dichloromethane and toluene showed no propensity of inter-conversion. XRPD analysis of samples after 7 and 14 days showed signal associated with the starting

material, with additional unique peaks present. (See Figure 13 (c)). The XRPD pattern of slurry study samples from n-heptane showed signals associated with the starting material, as well as other additional unique signals. The XRPD pattern of slurry samples from dichloromethane and toluene were consistent with spectra obtained after the thermal studies and the moisture sorption analysis. Analysis by DSC after 14 days revealed a small endotherm at 109°C, and a major endotherm at 167°C. HPLC using a gradient area percent assay did not show any significant degradation after the study.

5.6. Characterization of Diazoxide Salt of Hexamethyl Hexamethylene Diammonium Hydroxide (HHDADH)

[00676] The XRPD pattern of the HHDADH salt of diazoxide was analyzed, showing the material to be a crystalline solid. (See Figure 10(c)). Integration of the 1H NMR spectra was consistent with a 2:1 molar ratio of diazoxide to counter-ion (wherein the HHDADH counter-ion is divalent), and had the expected differences in the chemical shift of the aromatic and methyl resonances due to the change in the local environment of the aromatic system due to the presence of the choline counter-ion. (See Figure 14(c)). The λmax of the HHDADH diazoxide salt in acetonitrile measured by UV-vis is 296 nm, which is consistent with the sodium, potassium and choline diazoxide salts.

[00677] A summary of the characterization of the free form diazoxide and the potassium, sodium, choline and hexamethyl hexamethylene diammonium hydroxide salts of diazoxide are presented in Table 14.

Table 14: Summary of Characterization and Solubility for Diazoxide and Salts

Test	Free form	Potassium diazoxide salt	Sodium diazoxide salt	Choline diazoxide salt	HHDADH
XRPD	crystalline	crystalline	crystalline	crystalline	crystalline
FTIR	consistent	consistent	consistent	consistent	N/A
DSC	single endotherm at 330°C	multiple endotherms below 300°C	Single exotherm above 400°C	two endotherms below 200°C	N/A
UV in aqueous acetonitrile	268 nm 264 nm	265 nm 296 nm	271 nm 296 nm	268 nm 296 nm	N/A 296 nm

TGA	<1%	7.7 (monohydrate 6.7%)	<1%	<1%	N/A
Moisture Sorption	non-hygroscopic – no hysteresis	deliquescent – hemi/monohydrate	deliquescent – potential hemihydrate	deliquescent – no hysteresis	N/A
Solubility pH 2.0 pH 7.0 pH 12.0	 0.4 mg/mL 0.04 mg/mL 4.8 mg/mL	 9.9 mg/mL 14.4 mg/mL 43.0 mg/mL	 13.0 mg/mL 18.1 mg/mL 48.6 mg/mL	 28.2 mg/mL 41.5 mg/mL >293 mg/mL	N/A

[00678] Additional solubility studies were conducted for diazoxide free base form and diazoxide choline salt, as reported in Table 15. Each determination was carried out in duplicate, slurrying each sample in a 100 mM phosphate buffer solution, pH 7.00. Duplicate samples were then titrated to pH 7.0 and pH 8.8 using a 0.1 N phosphoric acid solution, followed by stirring for 18h at ambient temperature. After this time all samples were centrifuged, and the supernatant was diluted with mobile phase (MeCN/ Water.). Solubility was then obtained using a calibration curve for diazoxide by HPLC analysis. In Table 15, the term "Diazoxide, free from" refers to the free base of diazoxide; the term "Diazoxide, choline salt" is the choline salt of diazoxide as described herein; the term "Diazoxide, choline salt, milled" refers to the choline salt of diazoxide which has been milled by methods described herein. Table 15 shows that in 100mM phosphate buffer, pH 7, and with titration with 0.1N phosphoric acid to a pH of about 6.8 to 8.8, solubility is notably suppressed compared to solubility at pH 10-11. Furthermore, the diazoxide choline salts were found to have increased solubility when compared to the parent free base.

Table 15: Summary of Solubility for Diazoxide and Diazoxide Choline Salt.

Sample	**Sample amount (mg)**	**Phosphate buffer amount (mL)**	**1N H_3PO_4 (mL)**	**pH after titration with 0.1N H_3PO_4**	**pH after 18h slurry**	**Solubility [mg/mL]**
Diazoxide, free form	49.23	1	0.01	6.87	6.90	0.07
Diazoxide	47.64	1	---	7.30	7.31	0.07

Sample	Sample amount (mg)	Phosphate buffer amount (mL)	1N H_3PO_4 (mL)	pH after titration with 0.1N H_3PO_4	pH after 18h slurry	Solubility [mg/mL]
free form						
Diazoxide, choline salt	44.34	1	0.15	7.22	7.45	0.12
Diazoxide, choline salt	48.26	1	0.11	8.81	8.64	0.18
Diazoxide, choline salt, milled	45.35	1	0.14	7.32	7.36	0.12
Diazoxide, choline salt, milled	53.15	1	0.16	8.50	8.52	0.20
Diazoxide, choline salt	50.0	1	---	---	10.57	42.11
Diazoxide, choline salt, milled	50.0	3	---	---	10.52	32.88

"---" Indicates no titration was conducted.

6. Polymorphic Forms of Diazoxide Salts

[00679] Polymorphic forms of the salts of diazoxide, characterization, and preparation thereof are described.

6.1. Polymorphic Forms of the Choline Salt of Diazoxide

6.1.1. Demonstration of Preparation of Polymorphic Form B of the Choline Salt of Diazoxide

[00680] A 1-L round bottom flask was charged with diazoxide (5 g), MEK (750 mL) and choline hydroxide (5.25 g of 50 wt % solution in water), and heated to 77°C. The mixture was allowed to cool to approximately ~30 °C, and filtered to remove insoluble

brown residues. The filtrate was then concentrated under reduced pressure to afford yellow oil, which was dried in vacuo at 55°C and 30 in. Hg to afford approximately 7.8 g as a waxy solid. The solids were dried in vacuo at 55°C and 30 in. Hg to afford 7.13 g of the choline salt of diazoxide as a crystalline solid. Elemental analysis. Theoretical: C 46.77%, H 6.04% and N 12.59%. Measured: C 45.44%, H 5.98% and N 11.46%.

6.1.2. Characterization of the Polymorphic Form B of the Choline Salt of Diazoxide

[00681] The polymorphic Form B of the choline salt of diazoxide was analyzed by XRPD, DSC, and 1H NMR. As shown in Figure 16, the two identified polymorphic forms of the choline salt of diazoxide show different XRPD patterns. See Figure 16, wherein (a) shows the polymorphic Form A of the choline salt of diazoxide and (c) shows the polymorphic Form B of the choline salt of diazoxide.

[00682] As shown in Figure 17, 1H NMR of the polymorphic Form B of the choline salt of diazoxide shows no change from the polymorphic Form A.

[00683] Moisture absorption of the polymorphic Form B crystal structure of the choline salt of diazoxide performed from 0 – 80% relative humidity (RH) at 25°C showed the material to be hygroscopic as the sample absorbed over 14.5% at 70% relative humidity, and deliquesced at 80% RH. The XRPD pattern following the desorption analysis indicated that the sample remained in the Form B crystal structure during the hydration and subsequent desorption.

[00684] Solubility measurements performed at pH 2, 7, and 12 in 10 mM phosphate buffer at room temperature showed solubility of the Form B crystal structure of the choline salt of diazoxide to be 32.8 mg/mL, 80.1 mg/mL and 216 mg/mL, respectively. The XRPD pattern of the solids obtained after the solubility analysis (at pH 2 and 7) indicated that Form B of the choline salt of diazoxide was still present.

[00685] Slurry experiments were performed on each form to determine the propensity for conversion and to search for possible new and/or unique forms. Upon slurrying Form B in CH2Cl2, n-heptane, and toluene, form conversion was not observed.

6.1.3. Demonstration of Preparation of the Polymorphic Form A of the Choline Salt of Diazoxide from the Polymorphic Form B of the Choline Salt of Diazoxide

[00686] Approximately 20 mg of the Form B polymorph of the choline salt of diazoxide was added to approximately 1 mL of acetone and heated to approximately 55°C. The mixture was filtered while hot and placed in a refrigerator (4°C) for 16 hours. No precipitate was observed. The solvent was evaporated down to dryness using a gentle stream of nitrogen. The resultant solids were also dried in vacuo at room temperature and 30 in. Hg. XRPD analysis showed the salt had converted from the Form B polymorph to the Form A polymorph.

6.1.4. Characterization of the Polymorphic Form A of the Choline Salt of Diazoxide

[00687] Form A is an anhydrous crystalline form of diazoxide choline, with an endothermic event at approximately 165 °C in the DSC (see Figure 20). The XRPD pattern for Form A is unique compared to that of Form B as shown in Figure 21. FTIR (ATR) spectroscopy additionally indicates differences between the two forms. 1H NMR analysis affords a spectrum consistent with diazoxide and a 1:1 ratio of compound/counterion. NMR data also indicate that the magnetic environment of the diazoxide structure changes between free and polymorph forms, as evidenced by a movement in chemical shift of the aromatic and methyl proton resonances. In addition, the resonance due to the amine proton is not observed which suggests deprotonation in solution. Weight loss by TGA is less than 1% and may be due to residual solvent. The temperature of weight loss is above 100 °C which suggests that solvent may have been bound (i.e., solvate material). Moisture-sorption analysis conducted at 25 °C from 0 to 80% RH (adsorption) and 75 to 0% RH (desorption) shows Form A to be a hygroscopic solid, showing 2.4 wt % water at 60% RH. The sample was found to have deliquesced above 75% RH. In comparison, Form B is also hygroscopic and showed 7.4 wt % water at 60% RH and deliquesced at 80% RH. XRPD analysis following the moisture-sorption experiment affords a pattern consistent with Form A. Solubility studies conducted on both forms at pH 2, 7, and 12 in phosphate buffer showed differences, with Form A

APOTEX_EX1009_PGR2026-00034_Page 175

showing 28, 41, and >293 mg/mL respectively. Solubility concentrations were determined using area-percent calculations with HPLC calibration curves. Slurry experiments were performed on each form to determine propensity for conversion and to see if a unique form could be generated. Upon slurrying Form A in CH2Cl2, n-heptane, and toluene, conversion to Form B was observed after seven days. These results suggest that Form A is less thermodynamically stable under these conditions than Form B according to Ostwald's Rule of Stages.

6.1.5. Screening for Polymorphic Forms of Diazoxide Choline Salt.

[00688] A polymorph screening study of diazoxide choline salt was conducted with a series of crystallization and slurry conditions. As described herein, interconversion of diazoxide choline salt forms A and B was observed during this investigation. Each polymorphic form of diazoxide choline salt resulting from this study was characterized using the techniques and procedures described herein. A summary of characterization tests is listed in Table 16.

Table 16. Characterization of Forms A and B of Diazoxide Choline Salt In Screening Study

Experiment	Form A	Form B
XRPD*	Crystalline	Crystalline
DSC	Endotherm ≈165 °C	Endotherm ≈160 °C
TGA	<1%	≈1%
FTIR (ATR)**	Consistent w/ structure	Consistent w/ structure
^{1}H NMR	Consistent w/ 1:1 ratio	Consistent w/ 1:1 ratio
Moisture Sorption	Hygroscopic – deliquesced at 90% RH	Hygroscopic – deliquesced at 80% RH
Solubility	pH 2: 28 mg/mL pH 7: 41 mg/mL pH 12: >293 mg/mL	pH 2: 33 mg/mL pH 7: 80 mg/mL pH 12: 216 mg/mL
Thermal	Started to convert to Form B after 14 days at 60 °C	Stable after 14 days at 60 °C

Experiment	Form A	Form B
Slurries	Converted to Form B after 7 days in *n*-heptane, toluene, and CH_2Cl_2	Stable after 14 days in CH_2Cl_2, THF, and *t*-AmOH

*Major peaks (2-θ):

Form A (9.8, 10.5, 14.9, 17.8, 17.9, 18.5, 19.5, 22.1, 22.6, 26.2, 29.6, 31.2);

Form B (8.9, 10.3, 12.0, 18.3, 20.6, 24.1, 24.5, 26.3, 27.1, 28.9).

** Unique FTIR (ATR) absorbances (cm^{-1}):

Form A (2926, 2654, 1592, 1449, 1248);

Form B (3256, 2174, 2890, 1605, 1463, 1235).

6.1.5.1. Solubility Screen in organic solvents.

[00689] Diazoxide choline, prepared in MEK using choline hydroxide as 50 wt % solution in water (see above) displayed some solubility in the following solvents: acetonitrile, acetone, ethanol, IPA, MEK, DMF, and methanol. These solvents were chosen due to differences in functionality, polarity, and boiling points and their ability to dissolve diazoxide. Other solvents which showed poor ability to dissolve salts were used as antisolvents and in slurry experiments where some solubility was observed: dioxane, MTBE, EtOAc, IPAc, THF, water, cyclohexane, heptane, CH2Cl2, and toluene.

[00690] Solvents for crystallizations during screening were chosen based on the solubility screen summarized in Table 17. Crystallizations of diazoxide choline from all conditions afforded a total of two forms, A and B. Forms A and B were found to be anhydrous polymorphs of diazoxide choline. Form B was observed to be generated from most solvents used. It was difficult to isolate pure Form A on large scales (>50 mg) as conditions observed to produce Form A on a smaller scale (approximately 50 mg or less) were found to result in Form B or mixtures of both forms on larger scales. Based on room-temperature slurry experiments, anhydrous Form B was found to be the most thermodynamically stable form in this study. Form A readily converted to Form B in all slurry solvents utilized.

Table 17. Solubility Screen for Diazoxide Choline Salt

Solvent	Cmpd (mg)	Solvent (mL)	Conc. (mg/mL)	Temp. (°C)	Soluble
MeCN	1.7	0.25	>6.80	rt	Yes
Dioxane	1.4	5.00	0.28	55	No
Acetone	1.9	0.25	7.60	55	Yes
MTBE	2.4	5.00	0.48	55	No
EtOH	1.5	0.25	>6.00	rt	Yes
EtOAc	1.2	5.00	0.24	55	No
IPAc	1.4	5.00	0.28	55	No
IPA	1.8	0.25	7.20	55	Yes
THF	1.1	5.00	0.22	55	No
MEK	1.8	1.00	1.80	55	Yes
DMF	1.2	0.25	>4.80	rt	Yes
Water	2.0	5.00	0.40	55	No
MeOH	1.9	0.25	7.60	55	Yes
c-Hexane	2.0	5.00	0.40	55	No
Heptane	1.9	5.00	0.38	55	No
CH_2Cl_2	1.3	5.00	0.26	55	Partially
Toluene	1.4	5.00	0.28	55	No

6.1.5.2. Single-Solvent Crystallizations

[00691] Fast cooling procedure: Diazoxide (approximately 20 mg) was weighed out into vials and enough solvent (starting with 0.25 mL) was added until the material completely dissolved at elevated temperature. After hot filtration the vials were placed in a refrigerator (4 °C) for 16 hours. After the cooling-process the samples were observed for precipitates which were isolated by filtration. Vials not demonstrating precipitates were evaporated down to dryness using a gentle stream of nitrogen. All solids were dried

in vacuo at ambient temperature and 30 in. Hg.

[00692] Slow cooling procedure: Diazoxide (approximately 30 mg of choline salt) was weighed out into vials and enough solvent was added until the material went into solution at elevated temperature. After hot filtration the vials were then slowly cooled to room temperature at the rate of 20 °C/h and stirred at room temperature for 1–2 hours. All solids were dried in vacuo at ambient temperature and 30 in. Hg.

[00693] Based on the initial solubility study, seven solvents were selected for the fast-cooling crystallization: acetonitrile, acetone, ethanol, IPA, MEK, DMF, and methanol. Table 18 shows a list of the solvents that were used and the amount of solvent needed to dissolve the material. After the cooling-process precipitates were noticed in samples # 2, 3, 5, and 6, the solids were isolated by filtration. The other samples (# 1, 4, and 7) were evaporated down to dryness using a gentle stream of nitrogen. The diazoxide choline salts were found to be consistent with Form A by XRPD analysis for all solids with the exception of sample #2 (consistent with the freeform) and sample #5 (consistent with Form B with preferred orientation observed).

Table 18. Single-Solvent Crystallization of Diazoxide Choline Salt Using Fast-Cooling Procedure

Entry	Solvent	BP (°C)	Cmpd (mg)	Solvent Amt (mL)	Conc (mg/mL)	Temp. (°C)	Precipitate	Form
1	Acetone	56	21.0	1.00	21.00	55	No/Evap	A
2	MeOH	64	20.3	0.25	81.20	55	Yes	FF*
3	EtOH	78	21.3	0.25	85.20	62	Yes	A
4	MEK	80	19.6	1.25	15.68	75	No/Evap	A
5	MeCN	81	20.6	0.25	82.40	55	Yes	Unique
6	IPA	82	22.8	0.25	91.20	62	Yes	A
7	DMF	153	26.0	0.25	104.00	55	No/Evap	A

[00694] In accordance with the data obtained from fast-cooling experiments, four solvents which showed precipitation of solids were chosen for the slow-cooling

experiments: MeOH, EtOH, MeCN, and IPA (Table 19). All obtained analyzable solids of the choline salt were found to be consistent with Form B by XRPD with the exception of Entry #1 which was consistent with diazoxide freeform and Entry #2 which was not analyzable. Mother liquor of Entry #2 was concentrated to dryness and the residual solids were analyzed by XRPD and found to be Form B material. As a result of obtaining freeform material from the single-solvent crystallizations in methanol, three more alcohols were tested for the single-solvent crystallizations using fast- and slow-cooling procedures. Tables 20 and 21 provide a list of the solvents that were used and the amount of solvent needed to dissolve the material. XRPD patterns of the fast-cooling procedure showed freeform of diazoxide from isobutanol, Form B from isoamyl alcohol, and Form A from tert-amyl alcohol compared to the slow-cooling procedure, which afforded Form B material from all three solvents.

Table 19. Single-Solvent Crystallization of Diazoxide Choline Salt Using Slow-Cooling Procedure

Solvent	Boiling Point (°C)	Material Amount (mg)	Solvent Amount (mL)	Conc. (mg/mL)	Temp. (°C)	Precipitate	Form
MeOH	64	32.1	0.3	107.00	62	Yes	FF*
EtOH	78	33.3	0.3	111.00	75	Yes	NA**
MeCN	81	30.9	0.3	103.00	62	Yes	B
IPA	82	33.7	0.3	112.33	80	Yes	B

Table 20. Single-Solvent Crystallization of Diazoxide Choline Salt Using Fast-Cooling Procedure

Solvent	Boiling Point (°C)	Material Amount (mg)	Solvent Amount (mL)	Conc. (mg/mL)	Temp. (°C)	Precipitate	Form
i-BuOH	108	29.7	0.3	99.00	78	Yes	(sm)*
i-AmOH	130	29.6	0.3	98.67	82	Yes	B
t-AmOH	102	29.5	0.3	98.33	95	No/Evap	A

Table 21. Single-Solvent Crystallization of Diazoxide Choline Salt Using Slow-

Cooling Procedure

Solvent	Boiling Point (°C)	Material Amount (mg)	Solvent Amount (mL)	Conc. (mg/mL)	Temp. (°C)	Precipitate	Form
i-BuOH	108	33.0	0.3	110.00	92	Yes	B
i-AmOH	130	28.2	0.3	94.00	92	Yes	B
t-AmOH	102	29.0	0.4	72.50	92	Yes	B

[00695] The results of the choline salt single-solvent fast- and slow-cooling crystallizations (see Tables 19 to 21) indicated that Form A was more likely to be isolated with fast-cooling profiles and Form B with slow-cooling profiles.

6.1.5.3. Binary Solvent Crystallizations

[00696] Binary-solvent crystallizations of the choline salt were performed using four primary solvents (MeOH, EtOH, IPA, and MeCN) and nine cosolvents (MTBE, EtOAc, IPAc, THF, *c*-hexane, heptane, toluene, CH_2Cl_2, and dioxane) with a fast-cooling profile (*supra*). XRPD patterns showed that Form B was obtained from mixtures of MeOH with MTBE, EtOAc, IPAc, toluene, and dioxane. As shown in Table 22, Form A was obtained from mixtures of MeOH with THF and with CH_2Cl_2 after evaporating the solvent to dryness. The mixtures of MeOH with cyclohexane and heptane provided the freeform of diazoxide. All solids obtained from fast-cooling procedures with EtOH, IPA, and MeCN as primary solvents provided Form B material.

Table 22. Binary-Solvent Crystallizations of Choline Salt of Diazoxide Using Fast-Cooling Procedure and MeOH as a Primary Solvent

Diazoxide Amt (mg)	MeOH* (mL)	Antisolvent	Amount (mL)	Precipitate	Form
27.8	0.3	MTBE	1.4	Yes	B
30.7	0.3	EtOAc	6.0	Yes	B
32.0	0.3	IPAc	6.0	Yes	B
31.9	0.3	THF	6.0	No/Evap to Dry	A

Diazoxide Amt (mg)	MeOH* (mL)	Antisolvent	Amount (mL)	Precipitate	Form
29.5	0.3	*c*-Hexane	2.0	Yes (small)	FF**
30.2	0.3	Heptane	2.0	Yes	FF**
29.3	0.3	Toluene	6.0	Yes	B
32.0	0.3	CH_2Cl_2	6.0	No/Evap to Dry	A
28.8	0.3	Dioxane	6.0	Yes	B

* Solids were dissolved at 62 °C.

** Freeform of diazoxide.

[00697] Binary-solvent recrystallizations of the choline salt with the slow-cooling procedure were performed using two primary solvents (IPA and MeCN) and nine cosolvents (MTBE, EtOAc, IPAc, THF, c-hexane, heptane, toluene, CH2Cl2, and dioxane). All solids obtained from a slow-cooling procedure with IPA and MeCN as primary solvents provided Form B material based on XRPD analysis. The results of binary-solvent crystallizations indicated that Form B was the most thermodynamically stable form of diazoxide choline.

6.1.5.4. Binary Solvent Crystallizations Using Water as a Cosolvent

[00698] In an attempt to investigate the formation of hydrates of the choline salt, experiments was performed using fast- and slow-cooling procedures and water as a cosolvent.

[00699] The fast cooling procedure (supra) was used with the exception of using different primary solvents which were miscible with water: acetone, acetonitrile, DMF, IPA, i-BuOH, i-AmOH, and t-AmOH. Water was utilized in these crystallizations as a cosolvent. All solids obtained from the fast-cooling procedure with water as the cosolvent provided diazoxide freeform material by XRPD analysis.

[00700] To compare the results obtained from the fast-cooling procedure a set of experiments was performed using a slow-cooling procedure and water as a cosolvent. All obtained solids were analyzed by XRPD and afforded patterns consistent with diazoxide freeform. Without wishing to be bound by theory, these results suggest that the

conditions used for crystallization caused dissociation of the choline salt. A small amount of a second crop was obtained in each sample, but only two samples were analyzable by XRPD and indicated that the samples were freeform material. All mother liquors were evaporated to dryness and the residual solids were also analyzed by XRPD to afford patterns consistent with Form B of the choline salt.

6.1.5.5. Metastable Zone Width Estimation

[00701] Form B: To produce a robust process, an understanding of the solubility profiles of the various solid forms under consideration is required. From a practical standpoint, this involves the measurement of the metastable zone width (MSZW) of pure forms, whereby the saturation and supersaturation curves of the different forms are generated over a well defined concentration and temperature range. This knowledge can then be used to design a crystallization protocol that should ideally favor a selective crystal growth of the desired form.

[00702] Form B of diazoxide choline salt showed moderate solubility in a solvent mixture made of MeCN/MeOH/MtBE (10:1:12, volume ratios). The wide width of the metastable zone as shown in Table 23 gives many seeding options. During the MSZW measurement, aliquots from the crystallizing material were withdrawn and analyzed by XRPD to ensure that no form conversion occurred during the experiment. Indeed, the material remained unchanged during the test.

Table 23. Meta-Stable Zone Width For Form B Diazoxide Choline Salt in MeCN/MeOH/MtBE (10:1:12) (v/v).

Conc.| (mg/mL)	Temp. In (°C)	Temp. Out (°C)	Temp. Range (°C)
30.8	53.2	35.0	18.2
28.5	49.0	33.6	15.4
26.5	47.0	32.0	15.0
24.7	43.8	29.1	14.7
23.2	40.5	28.5	12.0

21.9	38.0	26.0	12.0

[00703] Form A: The metastable zone width for Form could not be estimated because this polymorphic form converted during the experiment to Form B.

6.1.5.6. Crystallization of Form A of Diazoxide Choline Salt

[00704] The choline salt of diazoxide (160.3 mg) was dissolved in 1 mL of IPA at 55 °C which was then passed through a Millipore 0.45 μM filter into a clean vial. This vial was placed in freezer a -20 °C overnight. Solids were not noticed and the flask was scratched with a micro-spatula. The vial was placed back in the freezer and nucleation was noticed after ten minutes. The solids were collected by vacuum filtration and washed with 1 mL of MtBE. The solids were dried in vacuo at 40 °C and 30 in. Hg to afford 70 mg (43.6% recovery) of Form A as determined by XRPD.

6.1.5.7. 500-mg Scale Crystallization of Form B of Diazoxide Choline Salt

[00705] The choline salt of diazoxide (524.3 mg) was dissolved in 3 mL of IPA at 78 °C and this solution was then cooled to 55 °C for the addition of MtBE. The MtBE (4 mL) was added until nucleation was observed. After nucleation the batch was allowed to cool to room temperature at a rate of 20 °C /h. The solids were collected by vacuum filtration and washed with 1 mL of MtBE. The solids were dried in vacuo at 40 °C and 30 in. of Hg to afford 426.7 mg (81.3% recovery) of Form B as determined by XRPD.

6.1.5.8. 2-g Scale Crystallization of Form B of Diazoxide Choline Salt

[00706] The choline salt of diazoxide (2.0015 g) was dissolved in 5.5 mL of IPA at 78 °C to afford a clear solution. This solution was passed through a Millipore Millex FH 0.45 μM filter. This solution was then cooled to 55 °C. MtBE was added in 1 mL portions, with a two minute interval between portions. Nucleation was noted after the second addition of MtBE. This suspension was allowed to cool to room temperature at a

rate of 20 °C /h and stirred at this temperature for 16 hours. The solids were collected by vacuum filtration and washed with 1 mL of MtBE. The solids were dried in vacuo at 40 °C and 30 in. of Hg to afford 1.6091 g (80.4% recovery) of Form B as determined by XRPD.

6.1.5.9. Detection of Form Impurities

[00707] Mixtures of diazoxide choline Forms A and B were prepared by adding a minor amount of Form A to Form B. Samples were lightly ground by hands with a mortar and pestle for approximately one minute. Samples were then analyzed by XRPD analysis. XRPD analysis was found to be suitable for detecting 5% of Form A in Form B.

6.2. Polymorphic Forms of the Potassium Salt of Diazoxide

[00708] A summary of characterization tests for three common crystalline forms of diazoxide potassium salt are listed in Table 24. Solvents for crystallizations were chosen based on the solubility screen summarized below. Crystallizations of diazoxide potassium salt from all conditions provided herein afforded a total of seven unique crystalline forms, A through G. Forms C, D, and F were found to be the most common during the crystallization screen, and were therefore scaled up for further characterization.

Table 24. Results Summary of Characterization Tests for Salt of Diazoxide Potassium

Experiment	Form C	Form D	Form F
XRPD*	Crystalline	Crystalline	Crystalline
DSC	187, 360 °C	130, 191, 352 °C	191, 363 °C
TGA	8.4%**	4.5%**	13.1%**
FTIR (ATR)*	Consistent w/ structure	Consistent w/ structure	Consistent w/ structure
^{1}H NMR	Consistent w/ structure**	Consistent w/ structure	Consistent w/ structure**
Moisture Sorption	Hygroscopic – deliquesced at 90% RH Hygroscopic – deliquesced at 90%	Hygroscopic – deliquesced at 90% RH	

Experiment	Form C	Form D	Form F
	RH		
Solubility	N/A	pH 2: 29 mg/mL pH 7: 33 mg/mL pH 12: 59 mg/mL	N/A
Thermal	Stable (7 days)	Stable (7 days)	Stable (7 days)
Slurries	Converted to Form D	Converted to Form D	Converted to Form D

*XRPD Major peaks (2-θ):

Form A (6.0, 8.1, 16.3, 17.7, 18.6, 19.1, 22.9, 23.3, 23.7, 24.7, 25.4, 26.1, 28.2, 29.6, 30.2);

Form B (8.5, 10.8, 16.9, 18.2, 21.6, 25.5, 26.1, 28.9);

Form C (5.7, 6.1, 17.9, 23.9, 25.1, 37.3);

Form D (5.7, 6.2, 8.1, 8.5, 8.8, 16.9, 18.6, 23.2, 24.5, 25.8, 26.1);

Form E (6.7, 7.1, 14.1, 21.2);

Form F (8.5, 9.0, 18.7, 20.6, 23.5, 27.5, 36.3);

Form G (5.2, 5.5, 13.1, 16.5, 19.3, 22.8, 24.8, 26.4,28.7, 3 4.1);

*Unique FTIR (ATR) absorbances (cm^{-1}):

Form A (1503, 1374, 1339, 1207, 1131, 1056, 771);

Form B (1509, 1464, 1378, 1347);

Form C (1706, 1208, 1146, 746);

Form D (1595, 1258, 1219, 890);

Form E (1550, 1508, 1268, 1101, 1006).

Form F (1643, 1595, 1234, 1145, 810).

Form G (1675, 1591, 1504, 1458, 1432, 1266, 999, 958, 905, 872).

** Data indicates a half-molar equivalent of acetone, water, or dioxane for Forms C, D, and F respectively.

[00709] Diazoxide potassium Forms C, D, and F were observed to be an acetone solvate, a hemihydrate, and a dioxane solvate of diazoxide potassium, respectively. Form C is an acetone solvate that was generated predominantly when acetone was used in the crystallization. Form D, a hemihydrate, was observed to be generated from most solvents used. Form F is a dioxane solvate generated when dioxane was used as an antisolvent. Forms A, B, E, and G were not commonly observed during the crystallization. Elemental analysis data indicated that the unique forms observed may be mixtures and/or have

residual solvent(s) present.

[00710] Based on room-temperature slurry experiments presented, Form D was found to be the most thermodynamically stable form of those discovered in this study. Forms C and F readily converted to Form D in all slurry solvents utilized. Without wishing to be bound by theory, since nonaqueous solvents were used, the material may have converted to the hemihydrate, Form D, upon removal from the solvent.

6.2.1. Demonstration of Preparation of Polymorphic Form A of the Potassium Salt of Diazoxide

[00711] The polymorphic Form A of the potassium salt of diazoxide was prepared as described above.

6.2.2. Demonstration of Preparation of the Polymorphic Form B of the Potassium Salt of Diazoxide

[00712] Diazoxide (2.95 g) was combined with 450 mL of methyl ethyl ketone and heated to approximately 77°C to dissolve the diazoxide. To the solution was added approximately 13 mL of 1M potassium hydroxide at a rate of approximately 20 mL/min, stirred and allowed to cool to room temperature. The solution was stirred at room temperature for ~16 h. The solvent was removed under reduced pressure, and the residual solids were dried in vacuo at 57 °C and 30 in. Hg to afford 3.7 g of the potassium salt.

6.2.3. Characterization of the Polymorphic Form B of the Potassium Salt of Diazoxide

[00713] The Form B polymorph of the potassium salt of diazoxide was analyzed by XRPD, and 1H NMR. Figure 18 shows the XRPD pattern of (a) the Form A polymorph of the potassium salt of diazoxide and (b) the Form B polymorph of the potassium salt of diazoxide.

[00714] The 1H NMR of the Form B polymorph of the potassium salt of diazoxide shows no change from the Form A polymorph.

6.2.4. Demonstration of Preparation of the Polymorphic Form A of the Potassium Salt of Diazoxide from Form B

[00715] Approximately 20 mg of the Form B polymorph of the diazoxide potassium salt was added to 2 mL of acetone and heated until the material completely dissolved at 55°C. The solution was hot filtered and placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 18(a).

6.2.5. Preparation of the Polymorphic Form C of the Potassium Salt of Diazoxide from Form B

[00716] Approximately 20 mg of the Form B polymorph of the diazoxide potassium salt was added to 6 mL of ethyl acetate and heated until the material completely dissolved at 75°C. The solution was hot filtered and placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 18(c).

6.2.6. Preparation of the Polymorphic Form D of the Potassium Salt of Diazoxide from Form B

[00717] Approximately 20 mg of the Form B polymorph of the diazoxide potassium salt was added to 0.3 mL of isopropyl alcohol and heated until the material completely dissolved at 62°C. The solution was hot filtered and placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 19(a).

6.2.7. Preparation of the Polymorphic Form E of the Potassium Salt of Diazoxide from Form B

[00718] Approximately 20 mg of the Form B polymorph of the diazoxide potassium salt was added to 2.5 mL of tert-amyl alcohol and heated until the material completely dissolved at 95°C. The solution was hot filtered and placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 19(b).

6.2.8. Preparation of the Polymorphic Form F of the Potassium Salt of Diazoxide from Form B

[00719] Approximately 20 mg of the Form B polymorph of the diazoxide potassium salt was added to 0.6 mL of acetonitrile and heated until the material completely dissolved at 80°C. The solution was hot filtered, 6 mL of dioxane was added, and the solution was placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 19(c).

6.2.9. Preparation of the Polymorphic Form G of the Potassium Salt of Diazoxide from Form B

[00720] Approximately 22.3 mg of the Form B polymorph of the diazoxide potassium salt was added to 0.5 mL of isoamyl alcohol and heated until the material completely dissolved at 73°C. The solution was hot filtered, 6 mL of isopropyl acetate was added, and the solution was placed in a refrigerator (4°C) for 16 hours. No precipitate was formed. The solvent was evaporated down to dryness using a gentle stream of nitrogen and the resultant solids were dried in vacuo at room temperature and 30 in. Hg. The solid was analyzed by XRPD to determine the physical form. See Figure 19(d).

[00721] As shown in Tables 25 and 26, both the solvent used for recrystallization and the rate of cooling (i.e., fast cooling vs. slow cooling during the recrystallization) effect the crystal structure obtained once the product is isolated.

Table 25. Fast cooling of Potassium Salt of Diazoxide in Various Solvents

Solvent	Cmpd (mg)	Solvent (mL)	Recovery (mg)	Form
THF	20.8	6.5	14.2	B
EtOAc	21.1	6.0	10.6	C
MeCN	21.2	0.5	n/a	C + D
IPA	22.7	0.3	n/a	D
Water	20.6	1.5	n/a	Free form diazoxide
tAA	21.9	2.5	16.7	E
IAA	19.7	0.3	n/a	D
DMF	21.6	0.3	15.9	G

Table 26. Slow cooling of Potassium Salt of Diazoxide in Various Solvents

Solvent	Cmpd (mg)	Solvent (mL)	Recovery (mg)	Form
EtOAc	20.4	6.0	7.1	D
MeCN	22.8	0.5	13.8	C
IPA	21.5	0.3	13.2	C
Water	20.9	1.5	1.2	Free form diazoxide
tAA	21.5	2.5	15.4	E
IAA	20.6	0.3	6.6	D

6.2.10. Polymorphs Obtained by Recrystallization of the Potassium Salt of Diazoxide in Binary Solvents

[00722] As shown in Tables 27 and 28, recrystallization of the potassium salt of diazoxide from a variety of binary solvent systems also demonstrated conversion of the potassium salt to an alternate form. Use of acetonitrile as the primary solvent is shown in Table 27 and use of acetone as the primary solvent is shown in Table 28. As shown in Table 27, recrystallization of the Form B polymorph of the potassium salt of diazoxide from acetonitrile using methyl tert-butyl ether, ethyl acetate, isopropyl acetate, tetrahydrofuran, c-hexane, heptane, toluene and dichloromethane as the secondary solvent all yielded the D Form polymorph of the potassium salt. Recrystallization from acetonitrile using dioxane as the secondary solvent yielded the F Form polymorph of the diazoxide salt of potassium.

Table 27. Recrystallization in Acetonitrile

Acetonitrile (mL)	Secondary solvent	Cmpd (mg)	Recovery (mg)	Form
0.6	MTBE	20.7	13.7	D
0.6	EtOAc	23.4	9.5	D
0.6	IPAc	20.0	13.3	D
0.6	THF	20.3	6.4	D
0.6	c-Hexane	20.4	9.6	D
0.6	Heptane	20.3	10.8	D
0.6	Toluene	23.6	16.1	D
0.6	Dichloromethane	21.3	12.7	D
0.6	Dioxane	20.7	12.6	F

[00723] As shown in Table 28, recrystallization of the Form B polymorph of the potassium salt of diazoxide from acetone using methyl tert-butyl ether, tetrahydrofuran, and c-hexane as the secondary solvent all yielded the Form A polymorph of the potassium

salt of diazoxide. Recrystallization from acetone using ethyl acetate, heptane, toluene and dichloromethane as the secondary solvent all yielded the C Form polymorph of the potassium salt. Recrystallization from acetone using isopropyl acetate as the secondary solvent yielded the D Form polymorph of the diazoxide salt of potassium. Recrystallization from acetone using dioxane as the secondary solvent yielded the F Form polymorph of the diazoxide salt of potassium.

Table 28. Recrystallization in Acetone

Acetone (mL)	Other Solvent	Amt (mg)	Recovery (mg)	Form
2.0	MTBE	20.2	13.9	A
2.0	EtOAc	21.6	4.8	C
2.0	IPAc	20.6	11.6	D
2.0	THF	20.9	12.0	A
2.0	c-Hexane	21.3	12.3	A
2.0	Heptane	20.6	12.7	C
2.0	Toluene	20.4	13.3	C
2.0	Dichloromethane	21.1	13.0	C
2.0	Dioxane	20.4	12.5	F

6.2.11. Screening for Polymorphic Forms of Diazoxide Potassium Salt.

[00724] A polymorphic screening study of diazoxide potassium salt was conducted with a series of crystallization conditions described below.

6.2.11.1. Solubility Screening for Polymorphic Forms of Diazoxide Potassium Salt.

[00725] Diazoxide potassium, prepared in MEK using 1 M potassium hydroxide solution in water, displayed some solubility in the following ten solvents: acetone, THF,

EtOAc, MEK, MeCN, IPA, water, *t*-AmOH, *i*-AmOH, and DMF. These solvents were chosen due to differences in functionality, polarity, and boiling points and their ability to dissolve diazoxide. Solvents affording poor to fair solubility were used as antisolvents in binary/ternary crystallizations as well as slurry studies. Table 29 summarizes the results of the solubility screen.

Table 29. Solubility of Diazoxide Potassium Salt in Various Solvent

Solvent	Cmpd Amt (mg)	Solvent (mL)	Conc. (mg/mL)	Temp. (°C)	Soluble
MeCN	1.7	2.00	0.85	55	Yes
Dioxane	1.4	5.00	0.28	55	No
Acetone	1.6	4.00	0.40	55	Yes
MTBE	1.8	5.00	0.36	55	No
EtOH	2.2	0.75	2.93	55	Yes
EtOAc	1.8	5.00	0.36	55	No
IPAc	1.7	5.00	0.34	55	No
IPA	2.1	1.00	2.10	55	Yes
THF	1.8	5.00	0.36	55	Partially
MEK	1.5	5.00	0.30	55	Partially
DMF	1.6	0.25	>6.40	rt	Yes
Water	1.5	5.00	0.30	55	No
MeOH	1.5	0.25	6.00	55	Yes
c-Hexane	1.5	5.00	0.30	55	No
Heptane	1.2	5.00	0.24	55	No
CH_2Cl_2	1.3	5.00	0.26	55	No
Toluene	1.4	5.00	0.28	55	No

6.2.11.2. Single-Solvent Screening for Polymorphic Forms of Diazoxide Potassium Salt.

[00726] Single-solvent crystallizations of potassium salt were performed using ten solvents: acetone, THF, EtOAc, MEK, MeCN, IPA, water, *t*-AmOH, *i*-AmOH, and DMF for the fast-cooling procedure and six solvents (EtOAc, MeCN, IPA, water, *t*-AmOH, and *i*-AmOH) for the slow-cooling procedures. The "fast" and "slow" cooling procedures were as described above. Four of the solvents were excluded from the slow-cooling experiments because they did not provide solids during fast-cooling experiments and needed to be evaporated to dryness. Tables 30 and 31 provide a list of the solvents that were used and the amount of solvent needed to dissolve the material. All solids were analyzed by XRPD to determine the physical form and six unique patterns (Forms A–E, G) were observed.

Table 30. Single-Solvent Crystallizations of Potassium Salt of Diazoxide Using Fast-Cooling

Solvent	BP (°C)	Cmpd Amt (mg)	Solvent Amt (mL)	Conc (mg/mL)	Temp (°C)	Precipitate	Form
Acetone	56	20.7	2.0	10.35	55	No/Evap	A
THF*	65	20.8	6.5	3.20	63	No/Evap	B
EtOAc	76	21.1	6.0	3.52	75	Yes	C
MEK	80	20.2	4.0	5.05	75	No/Evap	A
MeCN	81	21.2	0.5	42.40	80	Yes	C+D
IPA	82	22.7	0.3	75.67	62	Yes	D
Water	100	20.6	1.5	13.73	95	Yes	FF
t-AmOH	103	21.9	2.5	8.76	95	Yes	E
i-AmOH	130	19.7	0.3	65.67	73	Yes	D
DMF	153	21.6	0.3	72.00	RT	No/Evap	G

*Solids were not completely dissolved.

Table 31. Single-Solvent Crystallizations of Potassium Salt of Diazoxide Using Slow-

Solvent	BP (°C)	Cmpd Amt (mg)	Solvent Amt (mL)	Conc (mg/mL)	Temp (°C)	Precipitate	Form
EtOAc	76	20.4	6.0	3.40	75	Yes	D
MeCN	81	22.8	0.5	45.60	80	Yes	C
IPA	82	21.5	0.3	71.67	80	No/Evap	C
Water	100	20.9	1.5	13.93	95	Yes	FF
t-AmOH	103	21.5	2.5	8.60	95	Yes	E
i-AmOH	130	20.6	0.3	68.67	80	Yes	D

6.2.11.3. Binary-Solvent Screening for Polymorphic Forms of Diazoxide Potassium Salt.

[00727] Binary-solvent crystallizations of the potassium salt utilizing fast-cooling procedure were performed using MeCN, acetone, and isoamyl alcohol as primary solvents and the following nine cosolvents: MTBE, EtOAc, IPAc, THF, *c*-hexane, heptane, toluene, CH_2Cl_2, and dioxane. Table 32 is representative, employing acetonitrile as primary solvent. XRPD patterns from crystallizations using acetonitrile as a primary solvent were consistent with Form D with only one exception being the solids obtained from the mixture of MeCN/dioxane afforded a unique pattern (Form F) material.

Table 32. Binary-Solvent Crystallizations of Potassium Salt of Diazoxide Using Fast-cooling Procedure and McCN as a Primary Solvent

Cmpd Amt (mg)	McCN* (mL)	Anti-solvent	Amt (mL)	Precipitate	Form
20.7	0.6	MTBE	1.0	Yes	D
23.4	0.6	EtOAc	6.0	Yes	D
20.0	0.6	IPAc	3.0	Yes	D
20.3	0.6	THF	6.0	No/Evap.	D

				To ppt	
20.4	0.6	*c*-Hexane	2.0	Yes	D
20.3	0.6	Heptane	2.0	Yes	D
23.6	0.6	Toluene	1.0	Yes	D
21.3	0.6	CH_2Cl_2	1.0	Yes	D
20.7	0.6	Dioxane	6.0	Yes	F

* Solids were dissolved at 80 °C.

[00728] XRPD patterns from binary crystallizations with acetone as primary solvent employing a fast cooling procedure afforded four forms A, C, D, and F. Mixtures of acetone with MTBE, THF, and cyclohexane provided Form A material; Form C was obtained from the mixtures of acetone with EtOAc, heptane, toluene, and CH2Cl2. The mixture of acetone with IPAc afforded Form D, and the mixture of acetone with dioxane afforded Form F solids.

[00729] XRPD patterns from binary crystallizations with isoamyl alcohol as the primary solvent employing a fast cooling procedure afforded five forms: C, D, E, F, and G. Form C was obtained from crystallizations using MTBE and EtOAc as cosolvents; Form D was obtained from mixtures of isoamyl alcohol with heptane, toluene, and CH2Cl2; Form E was crystallized out of i-AmOH/THF and i-AmOH/cyclohexane; Form G was obtained from i-AmOH/IPAc and Form F was obtained from i-AmOH/dioxane. Form D was the most common form observed from the crystallizations, and Form F was observed only when dioxane was used as an antisolvent.

[00730] Binary solvent recrystallizations of the potassium salt with the slow-cooling procedure were performed using three primary solvents (MeCN, acetone, and i-AmOH) and eight cosolvents (MTBE, EtOAc, IPAc, c-hexane, heptane, toluene, CH2Cl2, and dioxane). All solids were analyzed by XRPD to determine the physical form. Two patterns were observed to be Forms C and D respectively with additional peaks present. Other crystallizations provided Forms D, C, or F. Form D was obtained from the following solvent mixtures: MeCN/MTBE, MeCN/IPAc, MeCN/toluene, MeCN/CH2Cl2

and also from the mixtures of i-AmOH with MTBE, IPAc, cyclohexane, heptane, and toluene. Form C was obtained from MeCN/heptane, i-AmOH/EtOAc, and mixtures of acetone with MTBE, EtOAc, IPAc, cyclohexane, heptane, toluene, and CH2Cl2. Form F was crystallized from the mixtures of MeCN/dioxane and i-AmOH/dioxane. The solvent mixture of MeCN/EtOH provided amorphous material. Elemental analysis results indicate that the forms observed may not be pure and/or have bound or residual solvents present. Forms C, D, and F were found to be the most common forms of the potassium salt isolated and based on the results, these forms were chosen for scale-up and further characterization. Differences were found in the XRPD patterns and FTIR spectra of the scale-up lots which were attributed to differences in impurity profiles, crystallinity, and form purity.

6.2.11.4. Characterization of Polymorphic Form C of Diazoxide Potassium Salt.

[00731] Form C of diazoxide potassium salt is an acetone solvate with a 2:1 ratio of diazoxide/solvent. It is a crystalline form of diazoxide potassium, with endothermic events at 187 and 360 °C in the DSC. The XRPD pattern for Form C is unique compared to all other forms observed. FTIR (ATR) spectroscopy showed differences between forms. 1H NMR spectra were found to be consistent with the structure of diazoxide with a half-molar equivalent of acetone present. NMR data also indicated that the magnetic environment of the diazoxide structure had changed evidenced by a movement in chemical shift of the aromatic and methyl proton resonances. In addition, the resonance due to the amine proton was not observed which suggested deprotonation in solution. Weight loss by TGA was 8.9%, consistent with a half-molar equivalent of acetone, and occurred near 180 °C consistent with the endotherm observed in the DSC experiment. Moisture-sorption analysis conducted at 25 °C from 0 to 90% RH (adsorption) and 85 to 0% RH (desorption) showed Form C to be a hygroscopic solid, showing approximately 47 wt % water at 90% RH which indicated the sample deliquesced. In comparison, Forms D and F (hemihydrate and dioxane solvate) showed approximately 26 and 22 wt % water at 90% RH respectively. XRPD analysis following the moisture-sorption experiment afforded a pattern consistent with Form D. Slurry experiments were performed on 50/50 mixtures of Forms C, D, and F to determine propensity for

conversion and to see if a unique form could be generated. Upon slurrying Form C mixtures in ethyl acetate, acetonitrile, and isopropanol conversion to Form D was observed in all solvents. Without wishing to be bound by theory, these results as well as conversion of Form F to Form D in these conditions suggest that Form D is more thermodynamically stable than Form C and F according to Ostwald's Rule of Stages. A thermal-stability experiment on Form C at 60 °C found the form to be stable. Conversion to another form was not observed.

6.2.11.5. Characterization of Polymorphic Form D of Diazoxide Potassium Salt.

[00732] Form D of diazoxide potassium salt is a hemihydrate. It is a crystalline form of diazoxide potassium, with endothermic events at 130, 191, and 352 °C in the DSC. FTIR (ATR) spectroscopy showed differences between forms. 1H NMR spectra were found to be consistent with the structure of diazoxide. NMR data also indicated that the magnetic environment of the diazoxide structure had changed evidenced by a movement in chemical shift of the aromatic and methyl proton resonances. In addition, the resonance due to the amine proton was not observed which suggested deprotonation in solution. Weight loss by TGA was 4.5%, consistent with a half-molar equivalent of water, and occurred near 110 °C consistent with the endotherm observed in the DSC experiment. Moisture-sorption analysis conducted at 25 °C from 0 to 90% RH (adsorption) and 85 to 0% RH (desorption) showed Form D to be a hygroscopic, showing approximately 26 wt % water at 90% RH. In comparison, Forms C and F (acetone and dioxane solvates) showed approximately 47 and 22 wt % water at 90% RH, respectively. XRPD analysis following the moisture-sorption experiment afforded a pattern consistent with Form D. Solubility studies were conducted at pH 2, 7, and 12 for Form D and showed 29, 33, and 59 mg/mL respectively. Solubility concentrations were determined using area-percent calculations with an HPLC calibration curve. Slurry experiments were performed on 50/50 mixtures of Forms C, D, and F to determine their propensity for conversion and to see if a unique form could be generated. Upon slurrying mixtures of Form D with Form C or Form F in ethyl acetate, acetonitrile, and isopropanol conversion to Form D was observed in all solvents.

APOTEX_EX1009_PGR2026-00034_Page 198

6.2.11.6. Characterization of Polymorphic Form F of Diazoxide Potassium Salt.

[00733] Form F of diazoxide potassium salt is a dioxane solvate with a 2:1 ratio of diazoxide/solvent. It is a crystalline form of diazoxide potassium, with endothermic events at 191 and 363 °C in the DSC. The XRPD pattern for Form F is unique compared to all other forms observed. FTIR (ATR) spectroscopy showed differences between forms. ^{1}H NMR spectra were found to be consistent with the structure of diazoxide with a half-molar equivalent of dioxane present. NMR data also indicated that the magnetic environment of the diazoxide structure had changed as evidenced by a movement in chemical shift of the aromatic and methyl proton resonances. In addition, the resonance due to the amine proton was not observed which suggested deprotonation in solution. Weight loss by TGA was 13.1%, consistent with a half-molar equivalent of dioxane, and occurs near 180 °C consistent with the endotherm observed in the DSC experiment. Moisture-sorption analysis conducted at 25 °C from 0 to 90% RH (adsorption) and 85 to 0% RH (desorption) showed Form F to be a hygroscopic solid, showing approximately 22 wt % water at 90% RH. In comparison, Forms C and D (acetone solvate and hemihydrate) showed approximately 47 and 26 wt % water at 90% RH, respectively. XRPD analysis following the moisture-sorption experiment afforded a pattern consistent with Form D. Slurry experiments were performed on 50/50 mixtures of Forms C, D, and F to determine their propensity for conversion and to see if a unique form could be generated. Upon slurrying mixtures of Form F with Form C and Form D in ethyl acetate, acetonitrile, and isopropanol conversion to Form D was observed in all solvents.

B. *In vivo* Obesity Testing

1. Obesity animal model

[00734] Formulations of salts of any of the compounds of Formulae I – IV prepared as described herein can be tested for efficacy in an animal model of obesity as described by Surwit et al. (*Endocrinology* **141**:3630-3637 (2000)). Briefly, 4-week-old B6 male mice are housed 5/cage in a temperature-controlled (22°C) room with a 12-h light, 12-h dark

cycle. The high fat (HF) and low fat (LF) experimental diets contain 58% and 11% of calories from fat, respectively. A group of mice are fed the HF diet for the first 4 weeks of the study; the remaining 15 mice are fed the LF diet. The mice assigned to the LF diet are maintained on this diet throughout the study as a reference group of lean control mice. At week 4, all HF-fed mice a reassigned to 2 groups of mice. The first group remains on the HF diet throughout the study as the obese control group. The remaining 3 groups of mice are fed the HF diet and administered the controlled release formulation of salts of any of the compounds of Formulae I – IV at about 150 mg of active per kg per day as a single dose administered by oral gavage. Animals are weighed weekly, and food consumption is measured per cage twice weekly until the diets are changed at week 4, whereupon body weight and food intake are determined daily. The feed efficiency (grams of body weight gained per Cal consumed) is calculated on a per cage basis. Samples for analysis of insulin, glucose, and leptin are collected on day 24 (4 days before the diets are changed), on day 32 (4 days after the change), and biweekly thereafter. In all cases food is removed 8 h before samples are collected. Glucose is analyzed by the glucose oxidase method. Insulin and leptin concentrations are determined by double antibody RIA. The insulin assay is based on a rat standard, and the leptin assay uses a mouse standard. At the termination of the study, a postprandial plasma sample is collected and analyzed for triglyceride and nonesterified fatty acid concentrations. After 4 weeks of drug treatment, a subset of 10 animals from each group is killed. The epididymal white adipose tissue (EWAT), retroperitoneal (RP) fat, interscapular brown adipose tissue (IBAT) fat pads, and gastrocnemius muscle are removed, trimmed, and weighed. The percent body fat is estimated from the weight of the epididymal fat pad. A subset of five animals from each group is injected i.p. with 0.5 g/kg glucose. At 30 min post injection, a plasma sample is collected and analyzed for glucose content by the glucose oxidase method.

2. Treatment of obesity in humans

[00735] Formulations of salts of any of the compounds of Formulae I – IV prepared as described herein can be tested for efficacy in obese humans, as described by Alemzadeh (Alemzadeh et al., *J Clin Endocr Metab* **83**:1911-1915 (1998)). Subjects consist of moderate-to-morbidly obese adults with a body mass index (BMI) greater than or equal to

APOTEX_EX1009_PGR2026-00034_Page 200

30 kg/m^2. Each subject undergoes a complete physical examination at the initial evaluation, body weight being measured on a standard electronic scale and body composition being measured by DEXA.

[00736] Before initiation of the study, all subjects are placed on a hypocaloric diet for a lead-in period of 1 week. This is designed to exclude subjects who are unlikely to be compliant and to ensure stable body weight before treatment. Up to 50 subjects are tested at each dosage of drug. Daily dosage is set at 100, 200, and 300 mg/day. The daily dose is divided into 2 doses for administration. The dose is administered as either one, two or three 50 mg capsules or tablets at each time of administration. Subjects are dosed daily for up to 12 months. Subjects are reviewed weekly, weighed, and asked about any side effects or concurrent illnesses.

[00737] Twenty-four-hour dietary recall is obtained from each subject. The dietary recalls are analyzed using a standard computer software program. All subjects are placed on a hypocaloric diet and encouraged to participate in regular exercise.

[00738] Before commencing, and after completion of the study, the following laboratory tests are obtained: blood pressure, fasting plasma glucose, insulin, cholesterol, triglycerides, free fatty acids (FFA), and glycohemoglobin, and measures of rate of appearance and oxidation of plasma derived fatty acids. Additionally, routine chemistry profiles and fasting plasma glucose are obtained weekly to identify those subjects with evidence of glucose intolerance and/or electrolyte abnormalities. Glucose is analyzed in plasma, by the glucose oxidase method.

[00739] Insulin concentration is determined by RIA using a double-antibody kit. Cholesterol and triglycerides concentrations are measured by an enzymatic method. Plasma FFA is determined by an enzymatic colorimetric method. SI was assessed by an iv glucose tolerance test (IVGTT) using the modified minimal model. After an overnight fast, a glucose bolus (300 mg/kg) was administered iv, followed (20 min later) by a bolus of insulin. Blood for determination of glucose and insulin is obtained from a contra lateral vein at -30, -15, 0, 2, 3, 4, 5, 6, 8, 10, 19, 22, 25, 30, 40, 50, 70, 100, 140, and 180 min. SI and glucose effectiveness (SG) are calculated using Bergman's modified minimal-model computer program before and after the completion of the study. Acute

APOTEX_EX1009_PGR2026-00034_Page 201

insulin response to glucose is determined over the first 19 min of the IVGTT, and the glucose disappearance rate (Kg) is determined from 8–19 min of the IVGTT. Body composition is measured by bioelectrical impedance before and at the completion of the study. Resting energy expenditure (REE) is measured by indirect calorimetry after an overnight 12-h fast, with subjects lying supine for a period of 30 min. Urine is collected over the corresponding 24 h, for measurement of total nitrogen and determination of substrate use, before and after the study.

3. Treatment of obesity in humans by coadministering diazoxide and phentermine

[00740] Evaluation of a prolonged co-administration of solid oral dosage form of salts of any of the compounds of Formulae I – IV thereof in combination with phentermine can be conducted in humans with moderate-to-morbid obesity and a body mass index (BMI) greater than or equal to 30 kg/m2. Each subject undergoes a complete physical examination at the initial evaluation, body weight being measured on a standard electronic scale and body composition by DEXA.

[00741] Before initiation of the study, all subjects are placed on a hypocaloric diet for a lead-in period of 1 week. This is designed to exclude subjects who are unlikely to be compliant and to ensure stable body weight before treatment. Up to 100 subjects are tested. Daily dosage of salts of any of the compounds of Formulae I – IV is set at 200 mg. The daily dose is divided into 2 doses for administration. The dose is administered as either a 100 mg capsule or a 100 mg tablet at each time of administration. Subjects are dosed daily for up to 12 months. Phentermine is administered as a single daily dose of 15 mg. Subjects are reviewed every two weeks, weighed, and asked about any side effects or concurrent illnesses.

[00742] All subjects are continued on a hypocaloric diet and encouraged to participate in regular exercise. Before commencing, and after completion of the study, laboratory tests as described in the example above are obtained.

[00743] 4. Prevention of diabetes in prediabetic humans

[00744] The example describes use of salts of any of the compounds of Formulae I –

APOTEX_EX1009_PGR2026-00034_Page 202

IV in a prediabetic subject to prevent the occurrence of diabetes. Subjects included in the study all have elevated risk of developing diabetes as measured by one of two methods. In a fasting glucose assay they have plasma glucose values between 100 and 125 mg/dl indicating impaired fasting glucose, or in an oral glucose tolerance test they have plasma glucose values between 140 and 199 mg/dl at 2 hours post-glucose load indicating they have impaired glucose tolerance. Treatment is initiated in any subject meeting either criteria. Treated subjects receive either 200 mg diazoxide per day as a 100 mg capsule or tablet twice per day or as two 100 mg capsules or tablets once per day. Placebo treated subjects receive either one placebo capsule or tablet twice per day or two placebo capsules or tablets once per day.

[00745] Treatment is continued for once year with OGTT or fasting glucose measured monthly.

5. A sustained release coformulation of diazoxide HCl and metformin HCl use to treat diabetic patients

[00746] A sustained release co-formulation of diazoxide HCl and metformin HCl is produced by forming a compressed tablet matrix that includes 750 mg of metformin HCl and 100 mg of diazoxide HCl. These active ingredients are blended with sodium carboxymethyl cellulose (about 5% (w/w)), hypromellose (about 25% (w/w), and magnesium stearate (<2% (w/w)). The compressed tablet is further coated with a combination of ethylcellulose (80% (w/w)) and methyl cellulose (20% (w/w)) as a thin film to control rate of hydration and drug release.

[00747] Type II diabetic patients are treated with the oral dosage form by administration of two tablets once per day or one tablet every 12 hours. Treatment of the patient with the drug is continued until one of two therapeutic endpoints is reached, or for so long as the patient derives therapeutic benefit from administration. The two therapeutic endpoints that would serve as the basis for the decision to cease treatment include the patient reaching a Body Mass Index (BMI (kg/m2)) between 18 and 25 or the re-establishment of normal glucose tolerance in the absence of treatment. The patient is monitored periodically for (a) glucose tolerance using an oral glucose tolerance test, (b) glycemic control using a standard blood glucose assay, (c) weight gain or loss, (d)

progression of diabetic complications, and (e) adverse effects associated with the use of these active ingredients.

6. Prevention or treatment of weight gain in a patient treated with olanzapine

[00748] Pharmacotherapy for schizophrenia is initiated for a patient meeting DSM III-R criteria for schizophrenia. The patient is administered 10 mg of olanzapine (Zyprexa, Lilly) once per day. Adjunctive therapy to the patient for schizophrenia includes 250 mg equivalent of valproic acid as divalproex sodium (Depakote, Abbott Labs). Weight gain, dyslipidemia and impaired glucose tolerance, and metabolic syndrome are high frequency adverse events in patients treated with this combination of anti-psychotics. Weight gain, dyslipidemia, impaired glucose tolerance or metabolic syndrome are treated by the co-administration of a therapeutically effective dose of a KATP channel opener. The patient is treated with administration of 200 mg/day of salts of any of the compounds of Formulae I – IV as a once daily tablet formulation. Administration of salts of any of the compounds of Formulae I – IV continues until the weight gain, dyslipidemia, impaired glucose tolerance or metabolic syndrome is corrected or until treatment of the patient with olanzapine is discontinued. Dyslipidemia is detected by measuring circulating concentrations of total, HDL, and LDL cholesterol, triglycerides and non-esterified fatty acids. Impaired glucose tolerance is detected through the use of oral or IV glucose tolerance tests. Metabolic syndrome is detected by measuring its key risk factors including central obesity, dyslipidemia, impaired glucose tolerance, and circulating concentrations of key proinflammatory cytokines.

7. Comparison of Single Doses of Diazoxide Administered as Proglycem® Oral Suspension or as Diazoxide Choline Controlled-Release Tablets in Obese Subjects.

7.1 Experimental Design

7.1.1. Objective of Study.

[00749] Clinical studies employing a randomized, open-label, parallel protocol comparing the safety, tolerability and bioavailability (i.e., pharmacokinetics) in obese

subjects of Proglycem® (oral suspension) and diazoxide choline salt (controlled-release tablet) were conducted. The study evaluated the safety and tolerability of a single 200 mg does (approximately 2 mg/kg) of diazoxide. The study further compared the single dose pharmacokinetics of an oral suspension of the free base of diazoxide (Proglycem®) with a controlled-release tableted formulation of diazoxide choline salt under fasting conditions in obese subjects.

7.1.2. Rationale for Study.

[00750] Diazoxide choline was selected as an alternative molecule for the oral administration of diazoxide because of significantly greater aqueous solubility over diazoxide free base, rapid conversion to diazoxide base on exposure to an aqueous environment, and incompatibility with incorporation into sustained release formulations. The fate of diazoxide choline in an aqueous medium in vitro has been extensively characterized. Without wishing to be bound by theory, once solubilized from the controlled-release tablet formulation, prior to absorption, the salt hydrolyzes to the free base of diazoxide and choline hydroxide. This was extensively characterized using UV/Vis absorbance, and occurred at all physiological relevant pH values from pH 2.0 to pH 8.5. This hydrolysis occurred in deionized water and buffered aqueous solutions, and in polar solvents that contained trace amounts of water. Thus, diazoxide, as the free base, is the molecular form absorbed following oral administration of the choline salt of diazoxide to animals or humans. Serum and plasma assays used in TK and PK analysis measure diazoxide free base concentrations. Dissolution assays measure diazoxide free base. Differences in plasma concentration-time profiles of diazoxide will be dependent on the time course of release of diazoxide choline from the tablet matrix in the intestinal tract. Historically, both oral suspension and immediate release capsule formulations of diazoxide have been commercially available, marketed as Proglycem®. The oral suspension has been shown to be highly bioavailable and rapidly absorbed upon administration. Because of the difficulty in characterizing dissolution from the oral suspension, the in-vitro dissolution of Proglycem® capsules was compared under standardized conditions to that of diazoxide choline controlled-release tablets (50 mg and 200 mg).

APOTEX_EX1009_PGR2026-00034_Page 205

7.1.3. Inclusion Criteria.

[00751] Inclusion criteria for the present study included age 18 to 65 years old inclusive and BMI between 30 and 45 kg/m2, inclusive, and signed informed consent. Female participants were required to be either postmenopausal for at least 1 year, surgically sterile [bilateral tubal ligation, bilateral oophorectomy, or hysterectomy], or practicing a medically acceptable method of birth control. Inclusion criteria further included freedom from serious medical disorders involving the kidneys, digestive system, heart and blood vessels, lungs, liver, eyes, nerves, brain, skin, endocrine system, bones or blood. Subjects, other than their obese condition, were generally healthy as documented by medical history, physical examination, vital sign, 12-lead ECG, and clinical laboratory assessments. Characteristics of the subjects randomized in the study are provided in Table 33.

Table 33. Characteristics of subjects randomized in the study

Parameter	**Proglycem Oral Suspension (n=15)**	**Diazoxide Choline Controlled-Release Tablet (n=15)**
	Mean±SD (range)	**Mean±SD (range)**
Age (yr)	32.5±12.1 (18-56)	27.9±12.6 (19-54)
BMI (kg/m^2)	32.9±4.3 (30.2-44.8)	33.8±3.2 (31.4-42.1)
Gender (male/female)	9/6	7/8

7.1.4. Exclusion Criteria.

[00752] Exclusion criteria included treatment with an investigational drug within 28 days prior to dosing, presence or history of a clinically significant disorder, clinical laboratory test values outside of the accepted reference range, reactive screen for HBV, HCV, or HIV, use of any medication affecting body weight, lipid or glucose metabolism within 2 months, use of any systemic prescription medication from 14 days prior to screening to dosing except hormonal contraceptives, use of any drug known to induce or

inhibit hepatic drug metabolism from 28 days prior to screening until dosing, positive test for drug of abuse, current tobacco use, positive pregnancy screen, or pregnant or breastfeeding.

7.1.5. Randomization.

[00753] A total of 30 subjects were randomized in the present study. Fifteen subjects were randomized to each arm. For convenience, the 30 subjects were broken up into two cohorts. Cohort 1 checked into the clinic on October 12, 2006, was dosed on October 14, 2006, remained in the clinic for 72 hours following dose administration, returning at 96 and 120 hours after dose administration. Cohort 1 included 10 subjects randomized to receive Proglycem Oral Suspension and 5 subjects randomized to receive a Diazoxide Choline Controlled-Release Tablet. Cohort 2 checked into the clinic on October 14, 2006, were dosed on October 16, 2006, remained in the clinic for 72 hours following dose administration, returning at 96 and 120 hours after dose administration. Cohort 2 included 5 subjects randomized to receive Proglycem® Oral Suspension and 10 subjects randomized to receive a diazoxide choline controlled-release tablet. All subjects completed the study.

7.1.6. Dosing.

[00754] Subjects randomized to the Proglycem® Oral Suspension arm received a single 200 mg dose (4 mL) taken with 240 mL of room temperature water. Subjects randomized to the diazoxide choline controlled-release tablet arm received a single tablet containing 290 mg of diazoxide choline, which is equivalent to 200 mg of diazoxide. Doses were administered after an overnight fast, and fasting continued until approximately 4.25 hours after dose administration at which time a standardized meal was served.

7.1.7. Safety Monitoring.

[00755] Clinical laboratory tests including hematology, clinical serum chemistry and urinalysis were conducted at screening and at end of study. Hematology included

APOTEX_EX1009_PGR2026-00034_Page 207

measurements of hemoglobin, hematocrit, white blood cell count with differential, red blood cell count and platelet count. Clinical serum chemistry included evaluation of sodium, potassium, BUN, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase, AST, ALT, glucose, total cholesterol, HDL-cholesterol, and triglycerides. Urinalysis included pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrites, urobilinogen, leukocytes, and microscopic urine analysis if sample was dipstick positive. All adverse events were recorded. Vital signs were collected at screening, at one hour prior to dosing, and after dose administration at 1, 3, 6, 9, 12, 24, 48, 72 and 120 hours. Continuous two-lead cardiac monitoring (telemetry) was performed on subjects from 24 hours prior to dose administration (to establish baseline data) until 24 hours after dose administration.

7.1.8. Exploratory Endpoints.

[00756] Three exploratory endpoints were evaluated in the study. Blood glucose, insulin and non-esterified fatty acids (NEFA) were measured prior to dosing and at 1, 3, 6, 9, 12, 24, and 48 hours post dosing. Blood glucose and insulin levels were also assessed at end of study. Data collected at times 1, 3, 24, and 48 hours after dose administration were obtained under fasting conditions. The remaining data points were post-prandial measures. Samples for pharmacokinetic analysis to evaluate bioavailability were collected prior to dosing and after dose administration at 1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, 48, 72, 96, and 120 hours.

7.2. Results

7.2.1. Adverse Events.

[00757] The adverse events observed with each of the two formulations are summarized in Tables 34 and 35. With the single exception, a moderate headache requiring treatment with acetaminophen in a subject treated with Proglycem® Oral Suspension, all adverse events were mild. Both formulations appeared to be well tolerated in the study. One subject administered with diazoxide choline controlled-release tablet experienced mild loss of appetite. Because most obese and obese diabetic animal

APOTEX_EX1009_PGR2026-00034_Page 208

model studies of diazoxide show some reduction in feed consumption, loss of appetite was, therefore, considered part of the pharmacodynamic response to the drug in obese patients, rather than an adverse event.

Table 34. Adverse events (AE) in subjects administered Proglycem® Oral Suspension (n=15)

Adverse Event	Hours Post Dosing	si	Corrective Treatment	Outcome
Gastrointestinal				
Nausea[a]	2.75	Mild	None	Resolved
Neurological				
Headache	20.0	Moderate	Therapy required acetaminophen	Resolved
Headache[b]	6.25	Mild	None	Resolved
Dizziness[c]	1.0	Mild	Assisted to supine position	Resolved
Dizziness[c]	3.25	Mild	Assisted to supine position	Resolved
Dizziness[d]	1.0	Mild	Assisted to supine position	Resolved
Dizziness[d]	4.25	Mild	Assisted to supine position	Resolved
Miscellaneous				
Chills[b]	6.25	Mild	None	Resolved
Dermatitis from ECG patches[a]	4.75	Mild	None	Resolved

Number of subjects with at least one AE = 5, AEs followed by the same superscript letter are the same subject

Table 35. Adverse events (AE) in subjects administered Diazoxide Choline (200 mg Diazoxide) Controlled-Release Tablet (n=15)

Adverse Event	Hours Post Dosing		Corrective Treatment	Outcome
Gastrointestinal				
Nausea[e]	1.5	Mild	None	Resolved
Dry Heaves[e]	1.6	Mild	None	Resolved
Neurological				
Lightheaded	12.0	Mild	Assisted to supine position	Resolved
Dizziness[g]	20.0	Mild	None	Resolved
Miscellaneous				
Chills[f]	2.0	Mild	None	Resolved
Back Pain[f]	2.0	Mild	None	Resolved
Warm[g]	20.0	Mild	None	Resolved

Number of subjects with at least one AE = 4, AEs followed by the same superscript letter are the same subject

[00758] In addition, a randomized double-blind, placebo-controlled clinical trial was conducted in 24 healthy volunteers. They were randomized in a 2:1 ratio of treated to placebo. Treated subjects received 300 mg/day diazoxide administered as 3 tablets of the controlled release formulation U described in Table 2. The dose was administered once per day in the morning for a period of 7 days.

[00759] There were more adverse events reported per subject in the placebo controls than in the diazoxide choline treated arm (Table 35'). The majority of the events were mild. The frequency of the most common adverse events was identical in the diazoxide choline treated arm and in the controls.

Table 35. Adverse events (AE) in subjects administered Diazoxide Choline (300 mg Diazoxide) Controlled-Release Tablet or Placebo

	Adverse Event	
	Diazoxide choline	Placebo
Headache	4 (25%)	2 (25%)
Nausea	3 (19%)	2 (25%)
Dizziness	2 (13%)	1 (13%)
n	16	8

7.2.2. Clinical Chemistry.

[00760] A summary of fasting glucose, fasting insulin, NEFA, and serum sodium (Na), potassium (K) and creatinine level is provided in Table 36. Neither treatment resulted in significant changes in serum Na, K or creatinine from baseline to end of study. Treatment with diazoxide primarily impacts glucose-stimulated insulin secretion rather than basal insulin secretion. Fasting glucose levels increased slightly in the first 3 hours following dose administration. This increase was more pronounced in the Proglycem® Oral Suspension arm as compared to the diazoxide choline controlled-release tablet arm. These results are consistent with the differences in measured rates of dissolution from these formulations (see Table 5 herein) and the PK levels (see Figures 24 and 25 herein). In this study, there is no evidence for a reduction in fasting insulin after the administration of the single dose of diazoxide. There is also no evidence for a significant increase in fasting glucose as measured at 24 or 48 hours after dose administration. The most sensitive pharmacodynamic response measure is NEFA. Diazoxide treatment normally results in a short-term increase in NEFA, which returns to levels at or below baseline in about 6 hours. Both formulations showed this transient increase in NEFA. Treatment with Proglycem® Oral Suspension resulted in a statistically significant increase ($p<0.001$) in NEFA at 3 hours, which by 6 hours after dose administration had returned to levels well below baseline. Similarly the increase in NEFA at 3 hours after administration of diazoxide choline controlled-release tablets was statistically significant

APOTEX_EX1009_PGR2026-00034_Page 211

($p<0.0012$). NEFA levels in the diazoxide choline controlled-release tablet treated subjects also returned to levels well below baseline by 6 hours after dose administration.

Table 36. Fasting glucose, fasting insulin, non-esterified fatty acid (NEFA), serum sodium (Na), potassium (K) and creatinine of subjects

Parameter	**Proglycem Oral Suspension (n=15)**	**Diazoxide Choline Controlled-Release Tablet (n=15)**
Fasting Glucose (mg/dL)	**Mean±SD (range)**	**Mean±SD (range)**
Baseline	93±6 (83-104)	93±6 (83-108)
1 h post-dose	99.4±7 (89-117)	97.9±7 (92-117)
3 h post-dose	102.8±6 (93-113)	98.0±7 (90-112)
24 h post-dose	97±5 (87-106)	98±8 (88-118)
48 h post-dose	92±5 (84-103)	93±5 (83-104)
Fasting Insulin (µIU/ml)		
Baseline	8.6±5.2 (2.9-24.1)	9.9±3.3 (4.4-16.3)
24 h post-dose	12.0±8.9 (6.1-41.9)	11.1±3.3 (5.5-17.0)
48 h post-dose	9.5±5.7 (4.0-28.9)	11.1±2.9 (5.3-17.8)
NEFA (µmol/L)		
1 h post-dose	0.36±0.11 (0.21-0.65)	0.34±0.14 (0.09-0.67)
3 h post-dose	0.53±0.13 (0.31-0.78)	0.55±0.19 (0.28-0.88)
6 h post-dose	0.13±0.07 (0.07-0.32)	0.11±0.04 (0.07-0.21)
24 h post-dose	0.36±0.10 (0.23-0.62)	0.40±0.18 (0.25-0.97)
48 h post-dose	0.38±0.13 (0.2-0.66)	0.39±0.15 (0.17-0.65)
Na (mEq/L)		
Baseline	138.0±2.0 (135-142)	138.1±2.6 (134-143)
End of Study	138.7±1.1 (137-141)	139.3±2.3 (135-144)

APOTEX_EX1009_PGR2026-00034_Page 212

K (mmol/L)		
Baseline	4.4±0.4 (3.9-5.5)	4.2±0.3 (3.8-5.1)
End of Study	4.4±0.3(3.9-5.2)	4.2±0.3(3.8-5.2)
Creatinine (mg/dL)		
Baseline	0.9±0.1 (0.7-1.1)	0.9±0.2 (0.7-1.4)
End of Study	1.0±0.1 (0.8-1.3)	0.9±0.2 (0.6-1.3)

[00761] All other clinical laboratory tests, including hematology, clinical serum chemistry, and urinalysis were within normal ranges for obese subjects.

7.2.3. Vital Signs.

[00762] A graph depicting sitting blood pressure at baseline and at various times following dose administration is provided in Figure 22. Neither treatment was associated with a sustained reduction or increase in blood pressure, nor was there any clear trend from baseline over 6, 9, 12, or 24 hours after dose administration. Pulse rates dropped incrementally from baseline levels in the period from dose administration to 3 hours post dosing (Figure 23). Pulse rates rose to levels equivalent to baseline or slightly above baseline in the period from 6 to 12 hours after dose administration, and remained at baseline levels from 24 hours after dose administration to the end of the study (Figure 23). Review of the baseline cardiac telemetry data for all subjects was conducted the morning of dosing. Based on the absence of clinical significant abnormalities, all subjects were allowed to proceed with dosing. The cardiac telemetry data from study time 0 to 24 hours after dose administration was evaluated. No clinically significant abnormalities (e.g. arrhythmias) were observed.

7.2.4. Preliminary Evaluation of Diazoxide Plasma Pharmacokinetics.

[00763] Compared to the Proglycem® Oral Suspension, the diazoxide choline controlled-release tablet had a 30% lower peak exposure (Cmax) and a 15% lower total

exposure (AUC) when the formulations were administered orally as 200 mg equivalents of diazoxide (Table 37). The timing of the peak plasma concentration (Tmax) occurred at a median time of 4 hr after administration of the oral suspension and 20 hr after administration of the diazoxide choline controlled-release tablet. The terminal half-life for diazoxide was similar with both formulations (29 hr for the oral suspension and 32 hr for the tablet). The two formulations had similar between-patient variability for Cmax and AUC. The most notable difference between the two formulations following a single dose was the lower, broader peak of the concentration-time profile of the tablet formulation (Figures 24 and 25). The peak concentration for the tablet averaged about 30% less and occurred about 16 hours later than the suspension. Figures 24 and 25 illustrate that the mean peak concentration was virtually unchanged between 12-hr and 24-hr post-dose following administration of the diazoxide choline controlled-release tablet formulation. Because of a more sustained release pattern, the tablet formulation was predicted to have a greater accumulation factor with chronic dosing than was the oral suspensions (3.96 vs. 2.84). Simulations of repeated once-daily dosing with the two formulations (assuming linear pharmacokinetics) predicted that the two formulations would have similar trough concentrations at steady-state (22-23 µg/mL) (see Figure 26).

Table 37. Summary Statistics for Plasma Diazoxide Pharmacokinetic Parameters after a Single Dose of Two Formulations

Diazoxide Choline Controlled-Release Tablets								
	Cmax	Tmax	AUC(0-24)	AUC(0-120)	AUC(0-∞)	λz	T1/2	Accum
	µg/mL	hr	µg•hr/mL	µg•hr/mL	µg•hr/mL	1/hr	hr	Factor
N	15	15	15	15	15	15	15	15
Mean	9.25	22.1	166	553	618	0.0242	31.9	3.96
Geomean	9.07	19.6	157	531	588	0.0229	30.2	3.74
St Dev	1.89	11.5	54	160	198	0.0085	10.6	1.48
SEM	0.49	3.0	14	41	51	0.0022	2.7	0.38
CV%	20.5%	52.0%	32.5%	28.9%	32.0%	35.2%	33.3%	37.5%

Median	9.08	20.0	164	518	586	0.0237	29.3	3.80
Min	5.53	8.00	74.6	261	274	0.0144	15.9	2.26
Max	13.2	48.0	266	842	1024	0.0437	48.3	7.84

Diazoxide Oral Suspension (Proglycem)								
	Cmax µg/mL	Tmax hr	AUC(0-24) µg•hr/mL	AUC(0-120) µg•hr/mL	AUC(0-∞) µg•hr/mL	λz 1/hr	T1/2 hr	Accum Factor
N	15	15	15	15	15	15	15	15
Mean	13.5	6.67	245	643	696	0.0256	28.6	2.84
Geomean	13.3	5.36	243	629	677	0.0249	27.9	2.79
St Dev	2.3	5.49	35	142	173	0.0068	6.3	0.57
SEM	0.6	1.42	9	37	45	0.0018	1.6	0.15
CV%	17.1%	82.3%	14.4%	22.1%	24.9%	26.7%	22.0%	20.0%
Median	13.3	4.00	245	620	671	0.0234	29.6	2.86
Min	10.1	2.00	183	464	488	0.0180	16.1	1.84
Max	18.0	24.0	314	926	1054	0.0430	38.5	4.25

7.3 Conclusions of Comparison of Single Doses of Diazoxide Administered as Proglycem® Oral Suspension or as Diazoxide Choline Controlled-Release Tablets in Obese Subjects.

[00764] The present clinical study on the comparison of a single dose of diazoxide choline controlled-release tablet with an equivalent dose of diazoxide administered as Proglycem® Oral Suspension in obese patients indicates that both formulations were well tolerated. With a single exception, moderate headache requiring treatment with acetaminophen in one subject treated with Proglycem®, all adverse events were mild.

Diazoxide choline controlled-release tablets appear to have a better CNS safety profile than Proglycem® Oral Suspension as evidenced by the absence of headaches and reduced rate of dizziness. Exploratory endpoints showed no detrimental impact. Preliminary pharmacokinetic analysis showed that compared to Proglycem® Oral Suspension, the diazoxide choline controlled-release tablet had a 30% lower peak exposure (Cmax) and a 15% lower total exposure (AUC) for the same 200 mg diazoxide equivalent dose. The timing of the peak plasma concentration (Tmax) occurred at a median time of 4 hr after administration of the oral suspension and 20 hr after administration of the diazoxide choline tablet. The terminal half-life for diazoxide is similar with both formulations (29 hr for the oral suspension and 32 hr for the tablet). The two formulations had similar between-patient variability for Cmax and AUC.

8. Increasing Insulin Sensitivity

[00765] Type II diabetes is characterized by increasing insulin resistance, ultimately followed in the progression of the disease by beta cell exhaustion and insulin dependence. Improvements in insulin sensitivity without contributing to beta-cell exhaustion are critical to management of type II diabetes. Likewise, preservation of beta-cell mass in newly diagnosed type I diabetics by KATP channel openers is dependent on the ability to provide beta-cell rest. In non-insulin dependent individuals, direct evidence of beta-cell rest is provided by reductions in fasting insulin. In insulin dependent individuals, insulin secretion is measured indirectly as basal c-peptide levels.

[00766] A controlled clinical study was conducted to evaluate the potential for Diazoxide Choline Controlled-Release Tablets administered once daily to improve insulin sensitivity and provide beta-cell rest as evidenced by reductions in homeostasis model assessment-insulin resistance (HOMA-IR) and reductions in fasting insulin. Up to 8 obese individuals with or without hypertension or dyslipidemia, who may also have impaired fasting glucose or impaired glucose tolerance were randomized to received no drug, or diazoxide equivalent doses of 100 mg/day, 200 mg/day or 300 mg/day for 14 days administered as Diazoxide Choline Controlled-Release Tablets. All subjects were placed on a reduced calorie diet. Fasting insulin was measured pre-dose and on day 7, when diazoxide was expected to have achieved steady state pharmacokinetics. Insulin

APOTEX_EX1009_PGR2026-00034_Page 216

resistance was reduced in a dose dependent manner by day 7 (Table 38). Reductions in fasting insulin were realized at all dose levels (Table 39). Thus, treatment with Diazoxide Choline Controlled-Release Tablets once per day result is rapid and dramatic improvements in insulin resistance which combined with the beta cell rest provided by reductions in fasting insulin result in preservation of beta cell mass and function while improving glycemic control.

Table 38 Changes in HOMA-IR in treated obese subjects

		Homa-IR				
		Pre-dose		Day 7		Percentage
Cohort	N	Mean	SEM	Mean	SEM	Change
Control	6	2.3	0.51	2.7	0.47	17%
100 mg/day	7	4.0	0.73	2.8	0.52	-30%
200 mg/day	5	3.1	0.80	2.1	0.66	-32%
300 mg/day	8	3.2	0.49	2.0	0.44	-38%

Table 39. Changes in fasting insulin in treated obese subjects

		Fasting Insulin (μIU/mL)				
		Pre-dose		Day 7		Percentage
Cohort	N	Mean	SEM	Mean	SEM	Change
Control	6	10.9	2.41	13.1	2.13	20%
100 mg/day	7	18.2	3.31	11.7	1.97	-36%
200 mg/day	5	13.7	3.45	9.1	2.72	-34%
300 mg/day	8	13.4	1.97	7.8	1.61	-42%

9. Relationship between PK parameters and weight and Body Mass Index (BMI)

[00767] The historic data relating PK parameters to weight and BMI for the Proglycem oral suspension product suggested that there was a strong negative relationship between diazoxide peak drug levels (Cmax) and diazoxide area under the curve (AUC) for single doses and the weight and body mass index of dosed patients. A strong negative relationship between these key PK parameters would result in the need to adjust dose by

patient in order to achieve consistent pharmacokinetic profiles in treated patients.

[00768] The relationship between Cmax and AUC and the weight and BMI of subjects receiving either Proglycem or Diazoxide Choline Controlled-Release Tablets was evaluated in a controlled clinical study. Fifteen overweight or obese subjects with weight ranging from 172 lbs to345 lbs and BMI ranging from 30.2 to 44.8 were randomized to receive a single 200 mg dose of diazoxide administered as Proglycem oral suspension. Fifteen overweight or obese subjects with weight ranging from 173 lbs to 288 lbs and BMI ranging from 31.4 to 42.1 were randomized to receive a single 200 mg dose of diazoxide administered as a Diazoxide Choline Controlled-Release Tablet. Circulating diazoxide levels were measured on samples collected prior to dosing and after dose administration at 1, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, 48, 72, 96, and 120 hours.

[00769] The correlation between weight and Cmax and AUC and between BMI and Cmax and AUC were evaluated within each of the dosed cohorts of subjects. There was a significant negative relationship between weight and AUC, weight and Cmax and between BMI and Cmax in the Proglycem treated subjects (Table 40). No such relationship existed between either weight or BMI and AUC or Cmax in the Diazoxide Choline Controlled-Release Tablet treated subjects (Table 41).

Table 40. Relationship between PK Parameters and Weight/BMI for Proglycem

	Proglycem Oral Suspension	
	AUC	Cmax
Wt	-0.56*	-0.75**
BMI	-0.31	-0.61*

(* $p<0.05$, ** $p<0.01$)

Table 41. Relationship between PK Parameters and Weight/BMI for Proglycem

	Diazoxide Choline Controlled-Release Tablet

	AUC	Cmax
Wt	-0.28	-0.23
BMI	-0.04	0.17

10. Clinical Studies of Diazoxide Treatment of Dyslipidemia

[00770] Seven obese subjects including those with and without dyslipidemia were dosed for 7 days with the equivalent of 400 mg per day of diazoxide administered as diazoxide choline controlled-release tablets QD (two tablets taken daily, each containing 200 mg equivalent diazoxide per tablet prepared as formula K' (Table 2)). Subsequently, they were withdrawn from treatment and monitored for an additional 7 days.

[00771] The results of lipid profile taken on day 7 of treatment and on day 7 of monitoring (post treatment) are shown as an average for the treated individuals in Table 42. On day 7 of treatment, treated individuals averaged 8.6% reduction in total cholesterol, an 8.7% increase in HDL cholesterol and a 57.6% reduction in circulating triglycerides. At the end of the 7 day minitoring period, there was a reversal of each of these impacts; Total cholesterol was only 6.9% below baseline, HDL cholesterol had returned to 1 mg/dL below baseline and circulating triglycerides were now 44.4% below baseline. The most dramatic changes were realized in a 46 year old male who started treatment with total cholesterol of 342 mg/dL, HDL cholesterol of 37 mg/dL and circulating triglycerides of 1221 mmol/L. Following treatment, this subject realized a reduction in total cholesterol of 60 mg/dL, increase in HDL cholesterol of 15 mg/dL and a reduction in circulating triglycerides of 980 mmol/L. Seven days after discontinuation of treatment, this individual's total cholesterol had risen by 11 mg/dL, HDL cholesterol had dropped by 4 mg/dL and circulating triglycerides had risen by 124 mmol/L mg/dL.

Table 42. Changes in blood lipid parameters in a group of treated obese dyslipidemic patients

Variable	Baseline	Day 7 of	7 Days post-

		treatment	dosing
Total Cholesterol (mg/dL)	233	213	217
HDL Cholesterol (mg/dL)	46	50	45
Triglycerides (mmol/L)	304	129	169

11. Clinical Studies of Diazoxide Treatment of Hypertriglyceridemia

[00772] Individual patients with elevated fasting triglycerides, with or without elevated total cholesterol or LDL-cholesterol, were treated with diazoxide, administered as Diazoxide Choline Controlled-Release Tablets for 7 to 49 days. All patients were dosed once per day for an uninterrupted period of time. The dosages were achieved by combinations of two tablets, a 50 mg diazoxide equivalent dose tablet of diazoxide choline made as formula J (Table 2) and a 200 mg diazoxide equivalent dose tablet of diazoxide choline made as formula K' (Table 2). Table 43 provides this information.

Table 43. Combinations of Diazoxide Choline Controlled-Release Tablets

Diazoxide equivalent dose	Tablets of J	Tablets of K'
100 mg	2	0
150 mg	3	0
200 mg	0	1
250 mg	1	1
300 mg	2	1
350 mg	3	1
400 mg	0	2

APOTEX_EX1009_PGR2026-00034_Page 220

[00773] Several patients (subjects 1-6 and 8-9) were dosed for day 7 or 14 days at the diazoxide equivalent doses indicated in Table 44. Patient 7 received a diazoxide equivalent dose of 150 mg for 5 days followed by 9 days at a diazoxide equivalent dose of 300 mg (Table 44). Several patients, subjects 10, 12 and 13, were treated for 7 days at a diazoxide equivalent dose of 150 mg, followed by 7 days at at a diazoxide equivalent dose of 200 mg, followed by 7 days at a diazoxide equivalent dose of 250 mg, followed by 7 days at a diazoxide equivalent dose of 300 mg, followed by 21 days at a diazoxide equivalent dose of 350 mg (Table 45). One patient, number 11, was treated by titrating the drug using diazoxide equivalent doses of 150 mg, 200 mg, 250 mg and 300 mg for 7, 14, 7 and 17 days, respectively. Patients were placed on a low fat diet at the start of treatment which continued to the end of treatment. A control subject group which did not receive diazoxide met the elevated triglyceride level of the patient group (above 150mg/dl measured in blood under fasting conditions) and were placed on the same low fat diet as the treatment group (Table 46).

[00774] Each subject's baseline circulating triglyceride level was calculated as the average of two measurements of fasting triglycerides taken approximately two weeks apart, with the second taken predose on the first day of treatment. Patients treated for 14 days or less had their fasting triglyceride levels measured on days 1, 3, 7, and 14 following the start of dosing. Patients treated for greater than 14 days had their fasting triglyceride levels measured on days 7, 14, 21, 28, 35, 42 and 49 following the start of dosing.

[00775] The controls, with a single exception with borderline high triglycerides, on average had higher fasting triglycerides at the end of the diet period than when they started.

Table 44. Fasting Triglyceride (mg/dl) Responses in Patients Treated for 7 or 14 days

		Study Day					
Subject	Dose	Baseline	1	3	7	14	Percent change

Baseline triglyceride between 150 and 200 mg/dl							
2	100 mg	173	139	124	85	109	-37%
3	100 mg	180	231	254	133	181	1%
1	200 mg	168	152	112	129	135	-20%
21	150mg/ 300mg	175	207	109	131	---	-25%
4	300 mg	181	159	124	113	106	-41%
22	300 mg	183	177	94	---	---	-48%
6	300 mg	187	188	162	188	157	-16%
5	400 mg	186	245	200	179	---	-4%
Baseline triglyceride between 200 and 500 mg/dl							
7	150mg/ 300mg	205.5	207	145	103	104	-49%
23	300 mg	265	152	101	---	---	-62%
24	300 mg	279	328	217	---	---	-22%
8	400 mg	273	284	207	174	---	-36%
Baseline triglyceride above 500 mg/dl							
9	400 mg	723	511	279	241	---	-67%

Table 45. Fasting Triglyceride (mg/dl) Responses in Patients Treated for 35 to 49 days

Subject	Baseline	7	14	21	28	35	42	49	Percent change
Subjects with baseline triglycerides between 150 and 200 mg/dl									
MSC	153	114	116	111	129	131	132	95	-38%
10	161	169	111	90	108	104	108	99	-39%

Subjects with baseline triglycerides between 200 and 500 mg/dl									
11	202	181	143	155	137	126	---	---	-37%
12	222	194	140	169	161	149	134	139	-37%
13	336	184	230	173	172	176	130	127	-62%

Table 46. Fasting Triglyceride (mg/dl) Responses in Patients on Low Fat Diet for 7 to 21 Days

		Study Day					
Subject	Baseline	1	3	7	14	21	Percent change
Baseline triglyceride between 150 and 200 mg/dl							
14	158	151	131	93	133	113	-28%
15	199	190	292	184	165	203	2%
Baseline triglyceride between 200 and 500 mg/dl							
16	212	246	194	274	255	246	16%
17	218	223	272	343	251	252	16%
18	234	227	161	229	208	287	23%
19	248	216	202	363	347	342	38%
20	299	425	308	502	275	---	-8%

[00776] Patients treated with diazoxide equivalent doses of 100 mg per day experienced reductions in fasting triglycerides of 38% at 7 days and 18% at 14 days of treatment. Patients treated with diazoxide equivalent doses of 200 mg per day experienced reductions in fasting triglycerides of 23% at 7 days and 20% at 14 days of treatment. Patients treated with diazoxide equivalent doses of 300 mg per day experienced reductions in fasting triglycerides of 18% at 7 days and 29% at 14 days of

treatment. Patients treated with diazoxide equivalent doses of 400 mg per day experienced reductions in fasting triglycerides of 50% at 7 days of treatment. Patients treated with diazoxide equivalent doses of 150 mg and 300 mg for 5 and 9 days, respectively experienced reductions in fasting triglycerides of 50% at 7 and 14 days of treatment. In patients titrated from 150 mg to 300 or 350 mg, fasting triglycerides were reduced by 21%, 31%, 38%, 38% and 49% at the end of treatment with diazoxide equivalent doses of 150 mg, 200 mg, 250 mg, 300 mg and 350 mg, respectively. The response to the drug tended to be more pronounced at any dose level at elevated baseline triglyceride levels. The most pronounced impact on fasting triglycerides were realized in subjects with baseline fasting triglyceride levels greater than 300 mg/dl. There was no consistency tendency for elevations in LDL-cholesterol in treated patients, even those starting with very high fasting triglyceride levels at baseline. For example, patient 8 and 9 had reductions in LDL-cholesterol of -8.6% and -15.3%, respectively. Patient 13, in contrast had a rise in LDL-cholesterol of 6.7% on day 21 and of 11% by day 42.

12. Randomized double-blind, placebo-controlled Diazoxide clinical trial in healthy volunteers

[00777] A randomized double-blind, placebo-controlled clinical trial was conducted in 24 healthy volunteers. They were randomized in a 2:1 ratio of treated to placebo. Treated subjects received 300 mg/day diazoxide administered as 3 tablets of the formulation U as described in Table 2. The dose was administered once per day in the morning for a period of 7 days. A number of lipid parameters were measured at baseline and again on day 7 including fasting triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and non-HDL cholesterol. Additionally, blood pressure was measured on a daily basis.

[00778] The lipid response to 7 days treatment with diazoxide choline is provided in table 47. Treatment of subjects with diazoxide choline for 7 days resulted in a reduction of triglycerides of 13.2%. In contrast, in the placebo treated arm there was an increase of 11.7%. There was a reduction in total cholesterol, LDL cholesterol and non-HDL cholesterol from baseline to day 7 in both arms. The reduction in the diazoxide choline treated arm was greater for each lipid parameter than the corresponding reduction in the

APOTEX_EX1009_PGR2026-00034_Page 224

placebo arm. There was no change in HDL cholesterol in the placebo arm, whereas there was an increase HDL cholesterol of 8.2% in the diazoxide choline treated arm. Seven days of diazoxide choline treatment was associated with a marked decrease in triglycerides, reductions in LDL, non-HDL and total cholesterol and a marked increase in HDL cholesterol in these subjects.

Table 47. Changes in lipid parameters associated with 7 days of treatment with diazoxide choline (300 mg diazoxide/day) or placebo.

Treatment	Lipid parameter	Baseline mean±SEM	Day 7 mean±SEM	Percent change from baseline
Diazoxide choline	Triglycerides (mg/dL)	76±7.8	66±3.9	-13.2%
	Total cholesterol (mg/dL)	175±7.5	171±6.4	-2.3%
	LDL cholesterol (mg/dL)	111±5.8	105±4.9	-5.4%
	HDL cholesterol (mg/dL)	49±3.3	53±2.9	8.2%
	non-HDL cholesterol (mg/dL)	126±6.6	118±5.5	-6.3%
Placebo	Triglycerides (mg/dL)	60±10.7	67±9.8	11.7%
	Total cholesterol (mg/dL)	171±13.7	167±9.9	-2.3%
	LDL cholesterol (mg/dL)	105±11.8	101±11.3	-3.8%
	HDL cholesterol (mg/dL)	53±4.4	53±3.7	0.0%
	non-HDL cholesterol (mg/dL)	117±12.1	114±10.6	-2.6%

[00779] There was no change in blood pressure in the placebo treated arm over the 7 days of treatment. In contrast, 7 days of diazoxide choline treatment resulted in a reduction in diastolic blood pressure of 6.2 mmHg. This reduction in diastolic blood pressure has been observed in other studies of diazoxide choline and has persisted through the end of 7 weeks of treatment. The blood pressure response to diazoxide choline treatment in two separate clinical studies is shown in table 48. TR002 utilized 435 mg diazoxide choline achieved from one tablet of formlation K' (290 mg diazoxide

choline) and two tabltes of formulation J (each 72.5 mg diazoxide choline). In PK008, the dose was administered as one and one ½ tablet formulation K'.

Table 48. Blood pressure changes in response to diazoxide choline treatment in two clinical studies.

Study	Diazoxide choline dose	Duration of treatment	Parameter	Baseline	End of treatment
EC004	435 mg	7 days	Systolic	113.2	113.5
			Diastolic	69.4	63.2
TR002	508 mg	49 days	Systolic	118.1	117.0
			Diastolic	77.7	67.0

13. Clinical study in Hypertriglyceridemic patients

[00780] A clinical study was conducted in 12 hypertriglyceridemic patients. Patients received 435 mg/day diazoxide choline (300 mg diazoxide/day equivalent dose), and were dosed once per day in the morning. They were divided into two groups, one initially receiving a formulation which has lower bioavailability (formulation U) and one receiving a formulation with higher bioavailability (formulation K'). After day 8 they were crossed over from one formulation to the other formulation. The dosages were achieved by ingesting 1.5 tablets of formula K' (200 mg diazoxide equivalent dose tablet of diazoxide choline) and 3 tablets of formula U (100 mg diazoxide equivalent dose tablet of diazoxide choline) (Table 2). Treatment continued for 16 days. The endpoint of the study was percentage change from baseline to day 15 of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol and non-HDL cholesterol. Lipid parameters were measured at baseline and on days 7 and 15. Blood pressure was collected at multiple time points..

[00781] Treatment of hypertriglyceridemic patients with diazoxide choline for 15 days resulted in marked reductions in triglycerides, total cholesterol, LDL cholesterol, and

APOTEX_EX1009_PGR2026-00034_Page 226

nonHDL cholesterol and a marked increase in HDL cholesterol. The bioavailability of the formulation directly contributed to the therapeutic response, with patients receiving the formulation having higher bioavailability showing improved response to treatment. In general, the longer the patient was treated with diazoxide choline, the greater the therapeutic response. Results are provided in Table 49.

Table 49. Lipid responses in diazoxide choline treated hypertriglyceridemic patients.

	Formulation U				Formulation K'			
Parameter	N	Day 7	N	Day 15	N	Day 7	N	Day 15
Triglycerides	6	-22%±14%	6	-24%±17%	6	-46%±10%	6	-39%±4%
Tot-C	6	-2%±2%	6	-9%±4%	6	-9%±3%	6	-13%±6%
LDL-C	6	1%±4%	6	-11%±4%	6	-7%±5%	6	-16%±11%
HDL-C	6	5%±13%	6	10%±5%	6	12%±4%	6	13%±4%
Non-HDL-C	6	-4%±4%	6	-14%±4%	6	-15%±4%	6	-20%±8%

[00782] Treatment with diazoxide choline also resulted in lower supine and standing blood pressure. At baseline patients in the study had supine systolic blood pressure of 121 mmHg and supine diastolic blood pressure of 69 mmHg. Seven days of treatment reduced supine blood pressure to 116/62.5, an average drop in supine blood pressure of nearly 6 mmHg. Similarly, at baseline patients in the study had standing blood pressure of 123/80 mmHg. After 7 days of treatment, this was reduced to 112/75.5 mmHg, an average drop in standing blood pressure of nearly 8 mmHg.

14. Administration with Food to Reduce Adverse Events

[00783] Two studies were conducted to confirm the benefit of administration of diazoxide choline with food to reduce the incidence of adverse events. In each study, the

APOTEX_EX1009_PGR2026-00034_Page 227

subjects received 435 mg/day of diazoxide choline administered either as one intact 290 mg tablet and two intact 72.5 mg tablets (one tablet of formlation K' (290 mg diazoxide choline) and two tabltes of formulation J) or as one intact 290 mg tablet (formulation K') and one half of a 290 mg tablet (formulation K') (latter resulting in a 145 mg dose). The dose was administered either on a fasted basis or within 15 minutes of a meal which included solid food.

[00784] In the study where the dose was administered while subjects were fasted, all of the 16 subjects receiving the drug experienced at least one adverse event. In contrast, in the study where the dose was administered with a meal, only 42% of 12 subjects experienced at least one adverse event. In particular, the incidence of gastrointestinal adverse events was substantially reduced including nausea, abdominal pain, abdominal distention, diarrhea, gastrointestinal reflux, and chest pain (table 50). Similarly, the incidence of headaches, edema, and various types of pain were also reduced or prevented when the drug was dosed with solid food. These results were observed despite the fact that there was no food effect on PK. At steady state, there is no difference in average circulating drug levels, Cmax or Tmax when the dose is administered with our without food. Administration with food significantly reduced the incidence of a wide range of adverse events without adversely affecting steady-state pharmacokinetics and therapeutic response. This conclusion is further supported by the data from CS04, in which diazoxide choline was administered with food, where the incidence of adverse events per subject was lower in the drug treated arm than in the placebo treated arm (table 35').

Table 50: Incidence of adverse events when dosed under fasted or fed conditions

Adverse Event	Incidence under fasted administration	Incidence under fed administration
Nausea	25%	8%
Abdominal pain	6%	0%
Abdominal distention	6%	0%
Diarrhea	6%	0%
GERD	6%	0%

Chest pain	18%	0%
Headache	68%	8%
Pain (extremity, back, neck, joint, etc.)	31%	0%
Swelling and edema	38%	17%

[00785] All patents and other references cited in the specification are indicative of the level of skill of those skilled in the art to which the invention pertains, and are incorporated by reference in their entireties, including any tables and figures, to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00786] One skilled in the art would readily appreciate that the present invention is well adapted to obtain the ends and advantages mentioned, as well as those inherent therein. The methods, variances, and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

[00787] Definitions provided herein are not intended to be limiting from the meaning commonly understood by one of skill in the art unless indicated otherwise.

[00788] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions

APOTEX_EX1009_PGR2026-00034_Page 229

embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00789] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

APOTEX_EX1009_PGR2026-00034_Page 230

THAT WHICH IS CLAIMED IS:

1. A method of treating a dyslipidema by (a) reducing an abnormally high total cholesterol level in a subject's blood, (b) reducing an abnormally high LDL cholesterol level in a subject's blood, (c) reducing an abnormally high VLDL cholesterol level in a subject's blood (d) reducing an abnormally high non-HDL cholesterol level in a subject's blood (e) reducing an abnormally high triglyceride level in a subject's blood and/or (b) raising an abnormally low HDL cholesterol level in a subject's blood, comprising administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, Formula V, Formula VI, Formula VII, and Formula VIII;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iv) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

2. The method of claim 1 wherein said subject suffers from nonalcoholic steatohepatitis.

3. The method of claim 1 wherein said subject is at risk for pancreatitis.

4. The method of claim 1 wherein said subject suffers or is at risk for metabolic syndrome.

APOTEX_EX1009_PGR2026-00034_Page 231

5. The method of claim 1 wherein said subject is obese.

6. The method of claim 1 wherein the total cholesterol level in said subject's blood is reduced.

7. The method of claim 1 wherein said formulation is administered once per 24 hours.

8. The method of claim 1 wherein said formulation is administered twice per 24 hours.

9. The method of any one of claims 1-8 wherein said K_{ATP} channel opener is diazoxide choline.

10. The method of claims one of claims 1-9 wherein the formulation further comprises an agent other than a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, Formula V, Formula VI, Formula VII, and Formula VIII, and wherein said other agent is effective at (a) reducing an abnormally high total cholesterol level in a subject's blood, (b) reducing an abnormally high LDL cholesterol level in a subject's blood, (c) reducing an abnormally high VLDL cholesterol level in a subject's blood (d) reducing an abnormally high non-HDL cholesterol level in a subject's blood (e) reducing an abnormally high triglyceride level in a subject's blood and/or (b) raising an abnormally low HDL cholesterol level in a subject's blood level.

11. The method of any one of claims 1-9 wherein said subject is co-administered an agent other than a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, Formula V, Formula VI, Formula VII, and Formula VIII, and wherein said other agent is effective at (a) reducing an abnormally high total cholesterol level in a subject's blood, (b) reducing an abnormally high LDL cholesterol level in a subject's blood, (c) reducing an abnormally high VLDL cholesterol level in a subject's blood (d) reducing an abnormally high non-HDL cholesterol level in a subject's blood (e) reducing an abnormally high triglyceride level in a subject's blood and/or (b) raising an abnormally low HDL cholesterol level in a subject's blood level.

APOTEX_EX1009_PGR2026-00034_Page 232

12. A method of reducing the level of circulating triglycerides in a subject with an elevated level of circulating triglycerides, comprising administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, Formula V, Formula VI, Formula VII, and Formula VIII;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iv) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

13. The method of claim 11 wherein the formulation further comprises a HMG-CoA reductase inhibitor.

14. The method of claim 12 wherein the HMG-CoA reductase inhibitor is a statin.

15. The method of claim 12 wherein the formulation further comprises a fibrate.

16. The method of claim 12 wherein the formulation further comprises niacin.

17. The method of claim 12 wherein the formulation further comprises a PPAR delta agonist or modulator.

18. The method of claim 12 wherein the formulation further comprises a thyroid receptor activator.

APOTEX_EX1009_PGR2026-00034_Page 233

19. The method of claim 12 wherein the formulation further comprises a MTP inhibitor.

20. The method of claim 12 wherein the formulation further comprises a squalene synthase inhibitor.

21. The method of any one of claims 12-20 wherein the K_{ATP} channel opener is diazoxide choline.

22. The method of any one of claims 12-20 wherein said subject is diagnosed with conditions selected from the group consisting of hypercholesterolemia, combined hyperlipidemia , endogenous hyperlipidemia, and hypertriglyceridemia.

23. The method of any one of claims 12-20 wherein said subject has elevated circulating total cholesterol.

24. The method of any one of claims 12-20 wherein said subject has elevated circulating LDL-cholesterol.

25. The method of any one of claims 12-20 wherein said subject has normal circulating total cholesterol.

26. The method of any one of claims 12-20 wherein said subject has normal circulating LDL-cholesterol.

27. The method of claim 12 wherein the patient has low circulating HDL-cholesterol.

28. The method of claim 12 wherein the patient has normal circulating HDL-cholesterol.

29. The method of any one of claims 1-28 wherein multiple administrations of the formulation are given over a period of days and the caloric intake of said subject during said period of days is substantially the same as before the beginning of said administrations.

30. A method of (a) reducing insulin dosing, (b) increasing glycemic control, or

APOTEX_EX1009_PGR2026-00034_Page 234

(c) delaying the loss of residual insulin secretion in a subject suffering from type I diabetes, comprising administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

31. The method of claim 30 wherein the formulation is administered once per 24 hours.

32. The method of claims 30 or 31 wherein the K_{ATP} channel opener is diazoxide choline.

33. A method of treating a subject suffering from type II diabetes, comprising co-administering to said subject a therapeutically effective amount of a formulation and an anti-diabetic drug that stimulates insulin secretion or is a short-acting antidiabetic drug, wherein the formulation is selected from the group consisting of:

i) a formulation comprising a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, Formula V, Formula VI, Formula VII, and Formula VIII;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

APOTEX_EX1009_PGR2026-00034_Page 235

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iv) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

34. The method of claim 33 wherein the formulation is administered once per 24 hours.

35. The method of claim 33 wherein the formulation and the short-acting anti-diabetic drug are co-administered separately, sequentially or simultaneously.

36. The method of claim 33 wherein the anti-diabetic drug is one or more agents selected from the group consisting of a DPP-IV inhibitor, a PPAR agonist or partial agonist, a GLP-1 analog or mimetic, a thiazolidinedione, a disaccharidase inhibitor, a fructose-1, 6-bisphosphatase inhibitor, and a sulfonylurea receptor antagonist.

37. The method of claim 36 wherein the sulfonylurea receptor antagonist is a meglitinide.

38. The method of claim 37 wherein the meglitinide is one or more agents selected from the group consisting of repaglinide, nateglinide, mitiglinide and pharmaceutically acceptable salts thereof.

39. The method of claim 36 wherein the DPP-IV inhibitor is one or more agents selected from the group consisting of sitagliptin, saxagliptin, vildagliptin, and pharmaceutically acceptable salts thereof.

40. The method of claim 36 wherein the GLP-1 analog or mimetic is one or more agents selected from the group consisting of exenatide, liraglutide, albugon, exendin-4 analogs and conjugates, and pharmaceutically acceptable salts and conjugates thereof.

41. The method of claim 36 wherein the thiazolidinedione or PPAR agonist or

APOTEX_EX1009_PGR2026-00034_Page 236

partial agonist is one or more agents selected from pioglitazone, rosiglitazone, troglitazone, tesaglitazar, muraglitazar, netoglitazar, LY518674, ragaglitazar, sipoglitazar, and pharmaceutically acceptable salts threof.

42. The method of claim 36 wherein the disaccharidase inhibitor is one or more agents selected from the group consisting of to acarbose, AO-128, and pharmaceutically acceptable salts thereof.

43. The method of claim 36 wherein the fructose-1,6-bisphosphatase inhibitor is one or more agents selected from the group consisting of phenyl phosphonates, benzimidazoles, CS-917, amlinoquinazolones, benzoxazole benzenesulfonamides , and pharmaceutically acceptable salts thereof.

44. The method of claim 36 wherein the formulation comprises 50 to 450 mg of diazoxide choline and is administered once per 24 hours as a controlled release oral formulation, and the anti-diabetic is a pharmaceutical composition comprising 5 to 10 ug exenatide and is administered twice per 24 hours, before meals.

45. The method of claim 44 wherein the controlled release oral formulation is a tablet and the exenatide is administered by subcutaneous injection.

46. The method of claim 36 wherein the formulation comprises 50 to 500 mg of diazoxide choline and is administered once per 24 hours as a controlled release oral formulation, and the short-acting anti-diabetic is a pharmaceutical composition comprising the meglitinide and is administered three times per 24 hours, before meals.

47. The method of claim 36 wherein the anti-diabetic is exenatide, nateglinide, mitglinide, repaglinide, and pharmaceutically acceptable salts or conjugates thereof.

48. The method of any one of claims 33-47 wherein the K_{ATP} channel opener is diazoxide choline.

49. The method of any one of claims 33-47 wherein the K_{ATP} channel opener is administered at bedtime.

50. A method of treating poly-cystic ovarian syndrome, comprising

APOTEX_EX1009_PGR2026-00034_Page 237

administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

51. The method of claim 50 wherein said treatment reduces circulating androgen levels or reestablishes normal ovulation cycles.

52. The method of claim 50 wherein the formulation is administered once per 24 hours.

53. The method of claims 50-52 wherein the K_{ATP} channel opener is diazoxide choline.

54. A method of treating a subject suffering from poly-cystic ovarian syndrome comprising co-administering to said subject a therapeutically effective amount of a formulation and an androgen inhibitor, a selective estrogen receptor modulator (SERM), or an ovulation inducing agent, wherein the formulation is selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

APOTEX_EX1009_PGR2026-00034_Page 238

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

55. The method of claim 54 wherein the formulation is administered once per 24 hours.

56. The method of claim 54 wherein the formulation and the androgen inhibitor, selective estrogen receptor modulator (SERM), or ovulation inducing agent are co-administered separately, sequentially or simultaneously.

57. The method of any one of claims 54-56 wherein the K_{ATP} channel opener is diazoxide choline.

58. A method of treating a subject suffering from poly-cystic ovarian syndrome comprising administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group

wherein all formulations further comprise therapeutically effective amount of an

APOTEX_EX1009_PGR2026-00034_Page 239

androgen inhibitor, a selective estrogen receptor modulator (SERM), or an ovulation inducing agent.

59. The method of claim 58 wherein the formulation is administered once per 24 hours.

60. The method of any one of claims 58-59 wherein the K_{ATP} channel opener is diazoxide choline.

61. A method of treating a subject suffering from hypertension consisting essentially of administering to said subject a therapeutically effective amount of a single oral agent wherein the oral agent is a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group.

62. The method of claim 61 wherein the formulation is administered once per 24 hours.

63. The method of claims 61 or 62 wherein the K_{ATP} channel opener is diazoxide choline.

64. A method of treating a subject suffering from hypoglycemia-associated autonomic failure comprising administering to said subject a therapeutically effective amount of a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel

APOTEX_EX1009_PGR2026-00034_Page 240

opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII;

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group; and

iv) a formulation comprising 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine.

65. The method of claim 64 wherein the formulation is administered once per 24 hours.

66. The method of claim 64 wherein the formulation is administered two times per 24 hours.

67. The method of claim 64 wherein the subject is suffering from type I diabetes.

68. The method of claim 64 wherein the subject is suffering from type II diabetes.

69. The method of claim 64 wherein the formulation is an oral or an intranasal formulation.

70. The method of any one of claims 64-69 wherein the K_{ATP} channel opener is diazoxide choline.

71. A formulation comprising diazoxide choline and about 1% to about 55% by weight of a polymer selected from the group consisting of polyethylene oxide and cellulose.

72. The formulation of claim 71 wherein the cellulose is selected from

APOTEX_EX1009_PGR2026-00034_Page 241

hydroxypropylmethyl cellulose, hydroxypropylcellulose, ethylcellulose, methylcellulose, carboxymethylcellulose, and a mixture of any two or more thereof.

73. The formulation of claim 71 wherein the polyethylene oxide is selected from PEO N750, PEO 303 or a mixture thereof.

74. A method of reducing the incidence of adverse effects resulting from orally administering a salt of a K_{ATP} channel opener comprising

causing to be orally injested a formulation selected from the group consisting of:

i) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula I, Formula II, Formula III and Formula IV, and a cation selected from the group consisting of an alkali metal and a compound comprising a tertiary amine or ammonium group;

ii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII; and

iii) a formulation comprising a salt, said salt comprising an anion of a K_{ATP} channel opener selected from the group consisting of Formula V, Formula VI, Formula VII and Formula VIII, wherein at least one substituent comprises an amino group; and,

a meal containing one or more solid food items sufficient to reduce the incidence of adverse effects resulting from the orally injested salt of a K_{ATP} channel opener.

75. The method of claim 74 wherein the formulation and the meal are taken within 15 minutes or less of each other.

APOTEX_EX1009_PGR2026-00034_Page 242

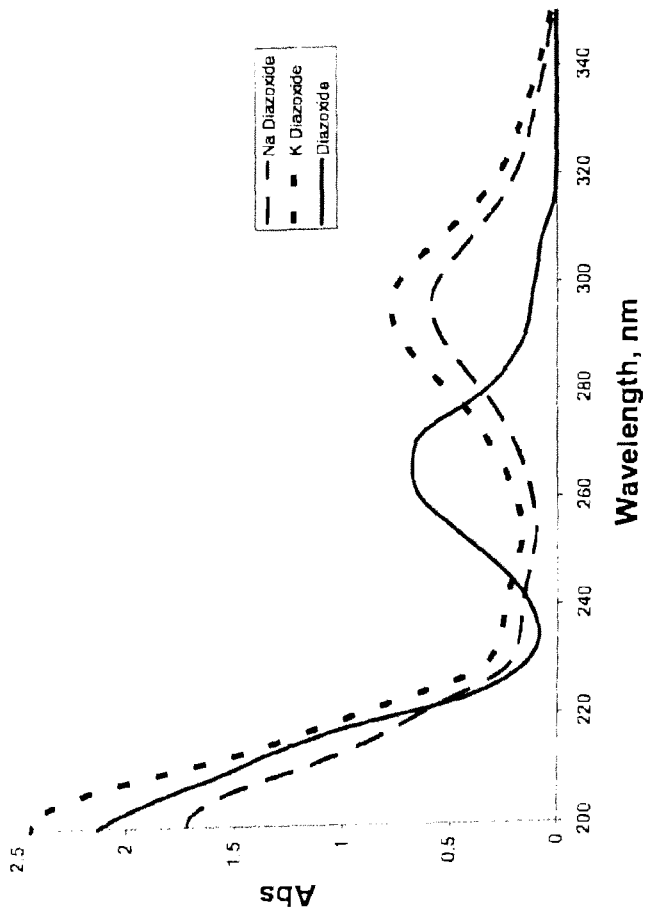
Na Diazoxide
K Diazoxide
Diazoxide
Abs
2.5
2
1.5
1
0.5
0
200
220
240
260
280
300
320
340
Wavelength, nm

FIG. 1

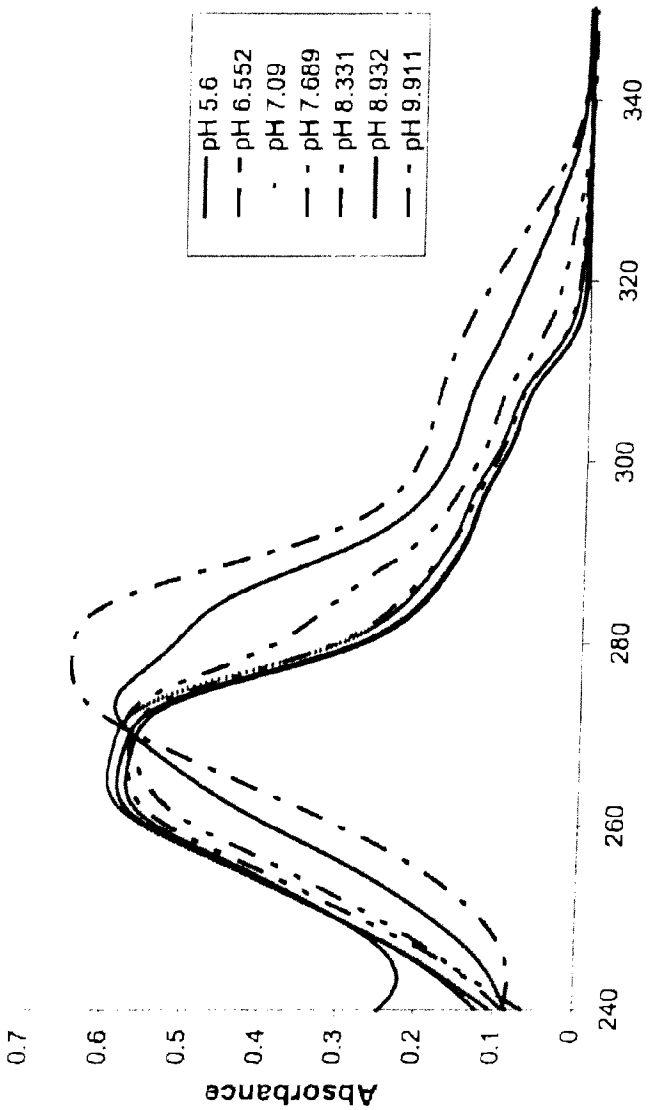
pH 5.6
pH 6.552
pH 7.09
pH 7.689
pH 8.331
pH 8.932
pH 9.911
Absorbance
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
240
260
280
300
320
340
Wavelength (nm)

FIG. 2

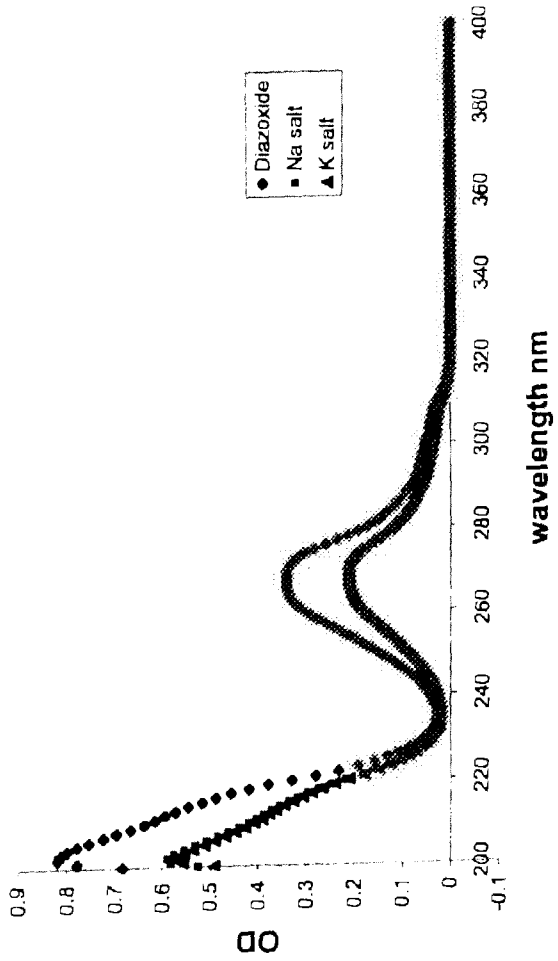
Diazoxide
Na salt
K salt
OD
wavelength nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
200
220
240
260
280
300
320
340
360
380
400

FIG. 3

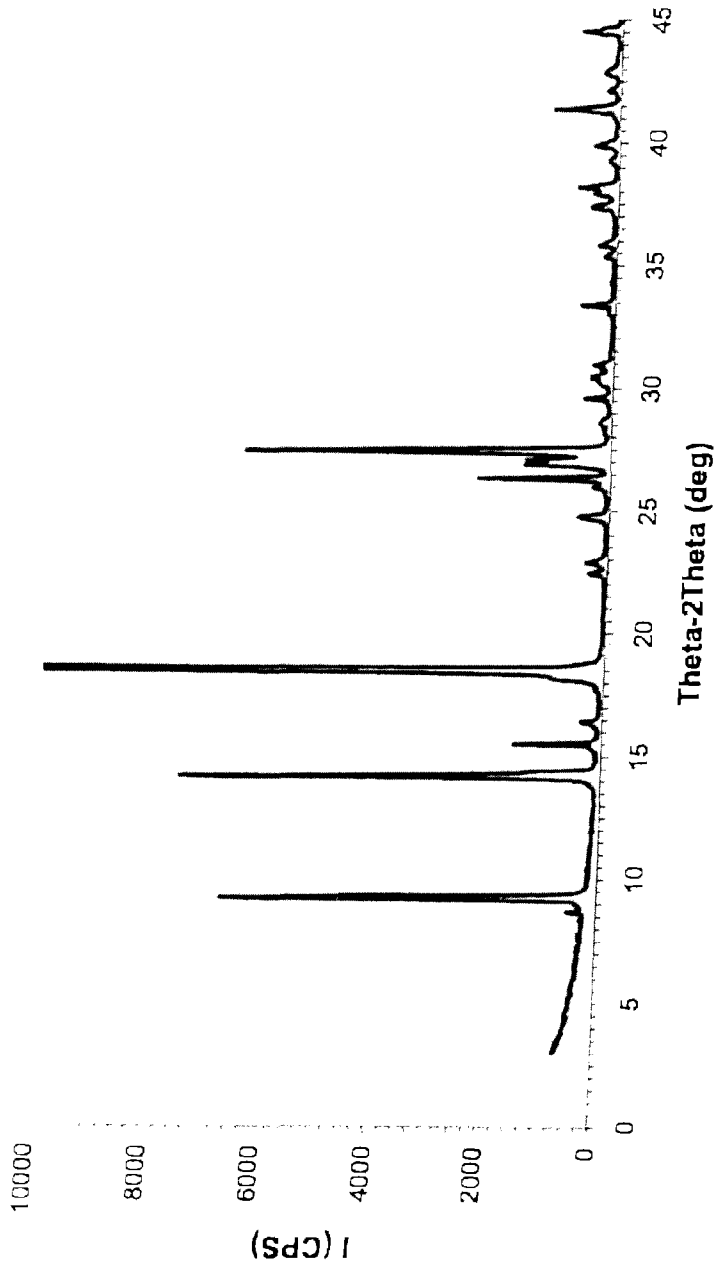
I (CPS)
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 4A

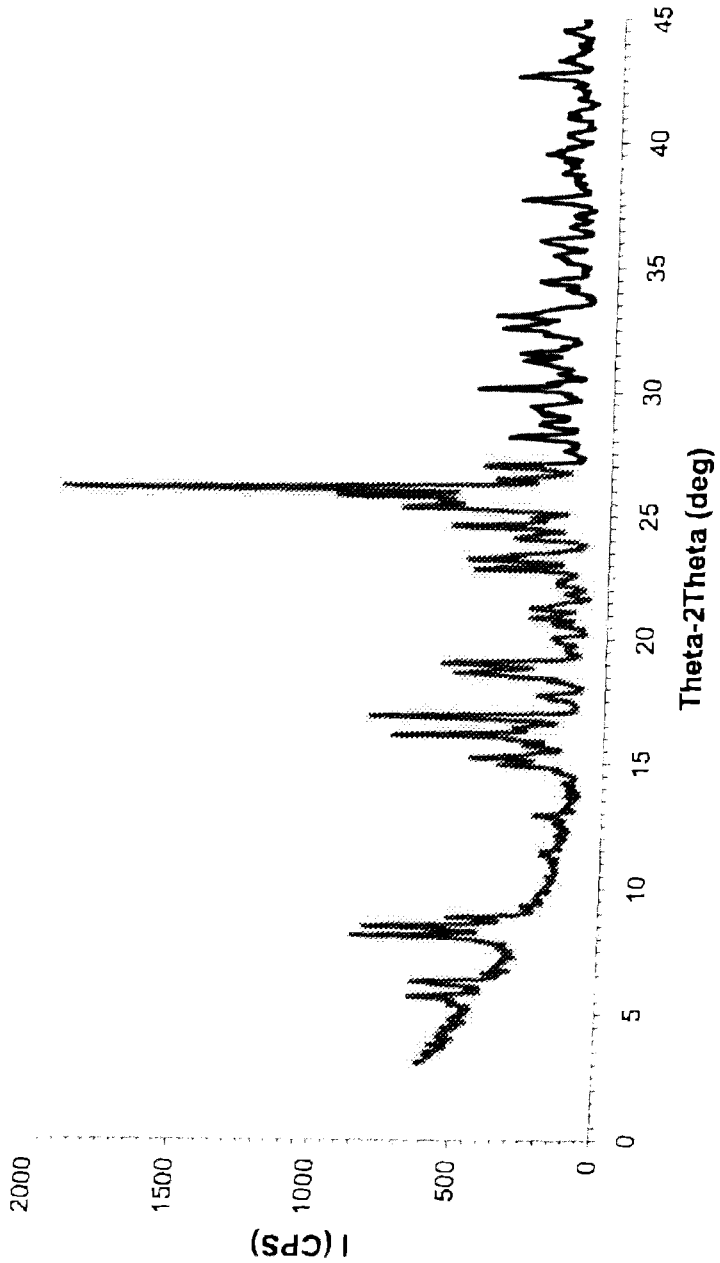
I (CPS)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 4B

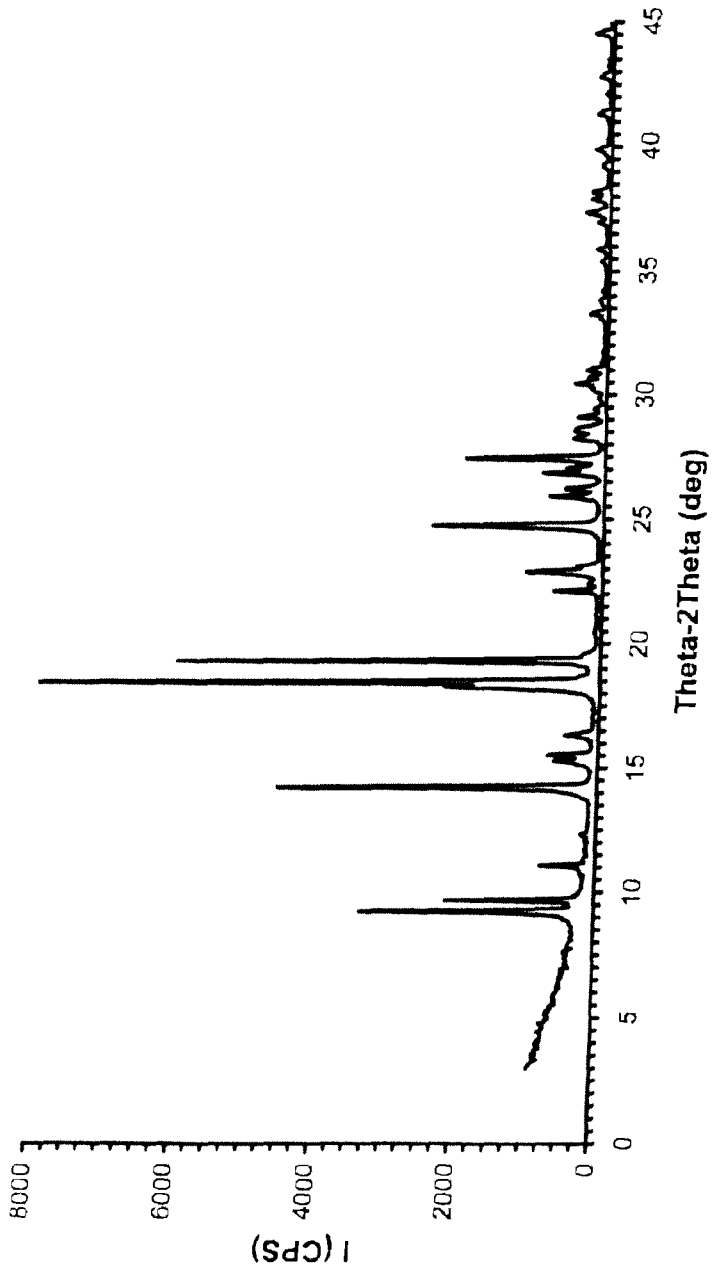
I (CPS)
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 4C

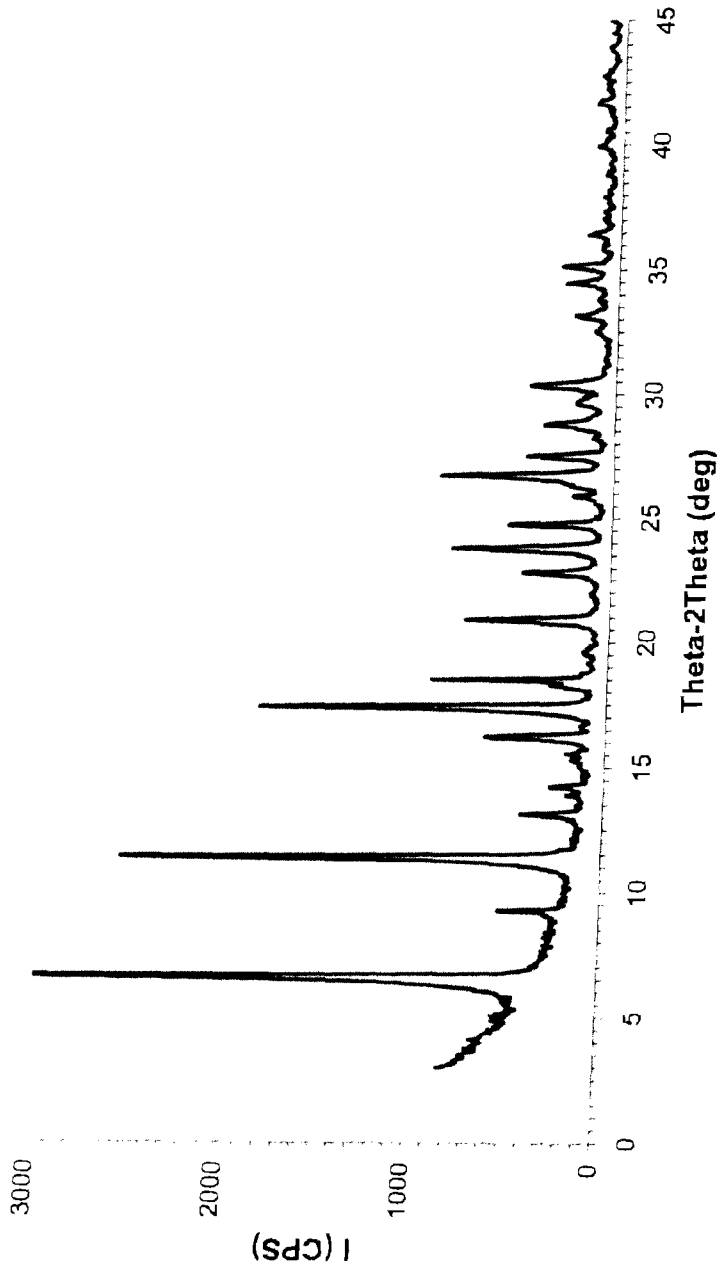

FIG. 4D

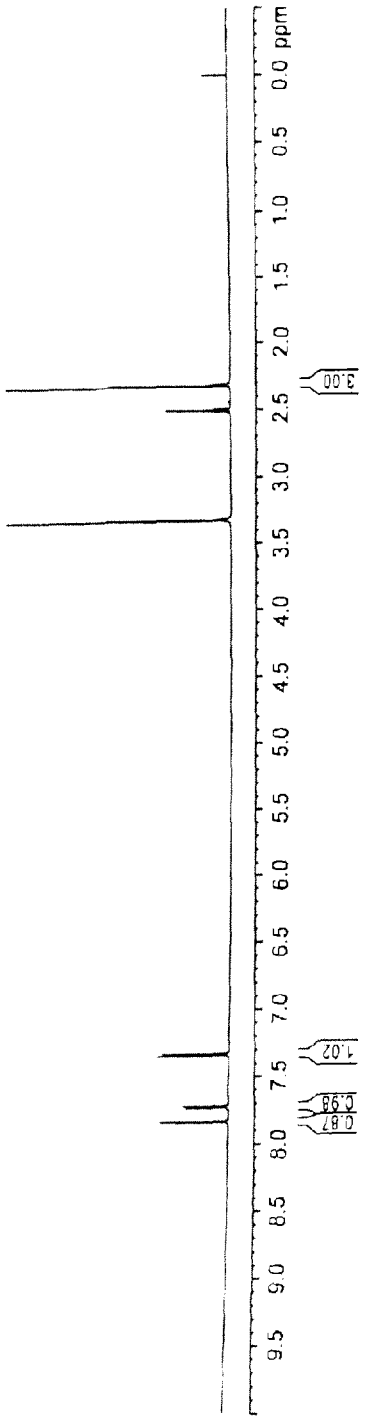
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0 ppm
3.00
1.02
0.98
0.87

FIG. 5A

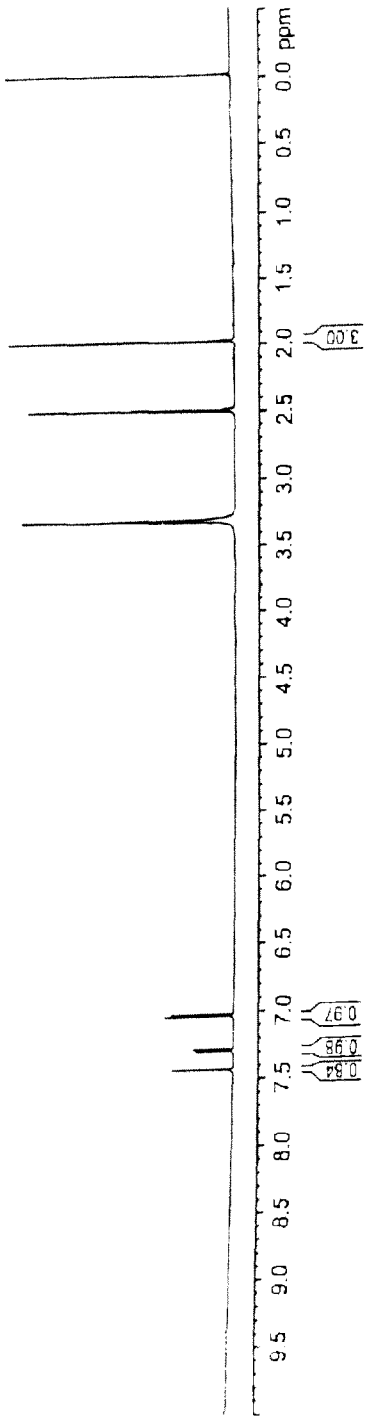
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0 ppm
3.00
0.97
0.98
0.84

FIG. 5B

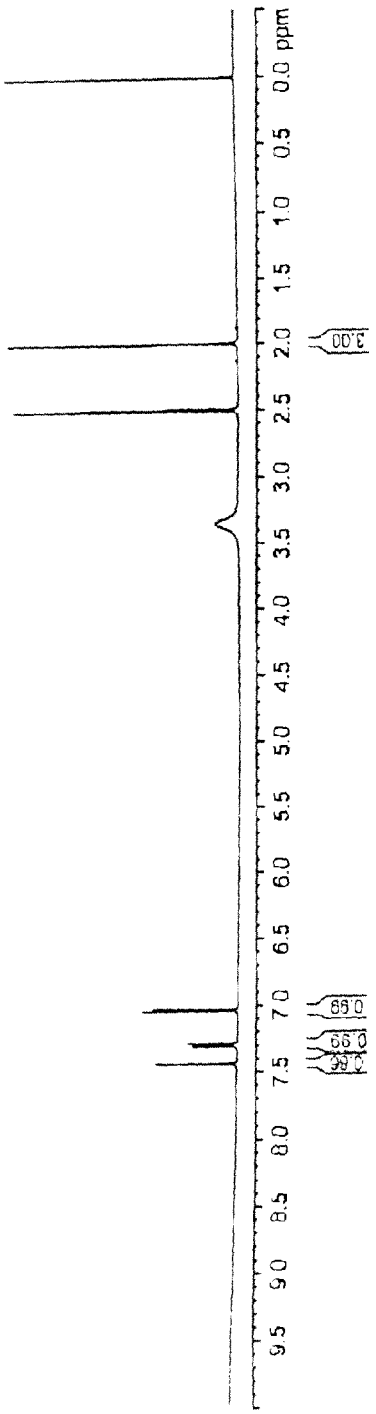
0.0 ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
3.00
0.99
0.99
0.99

FIG. 5C

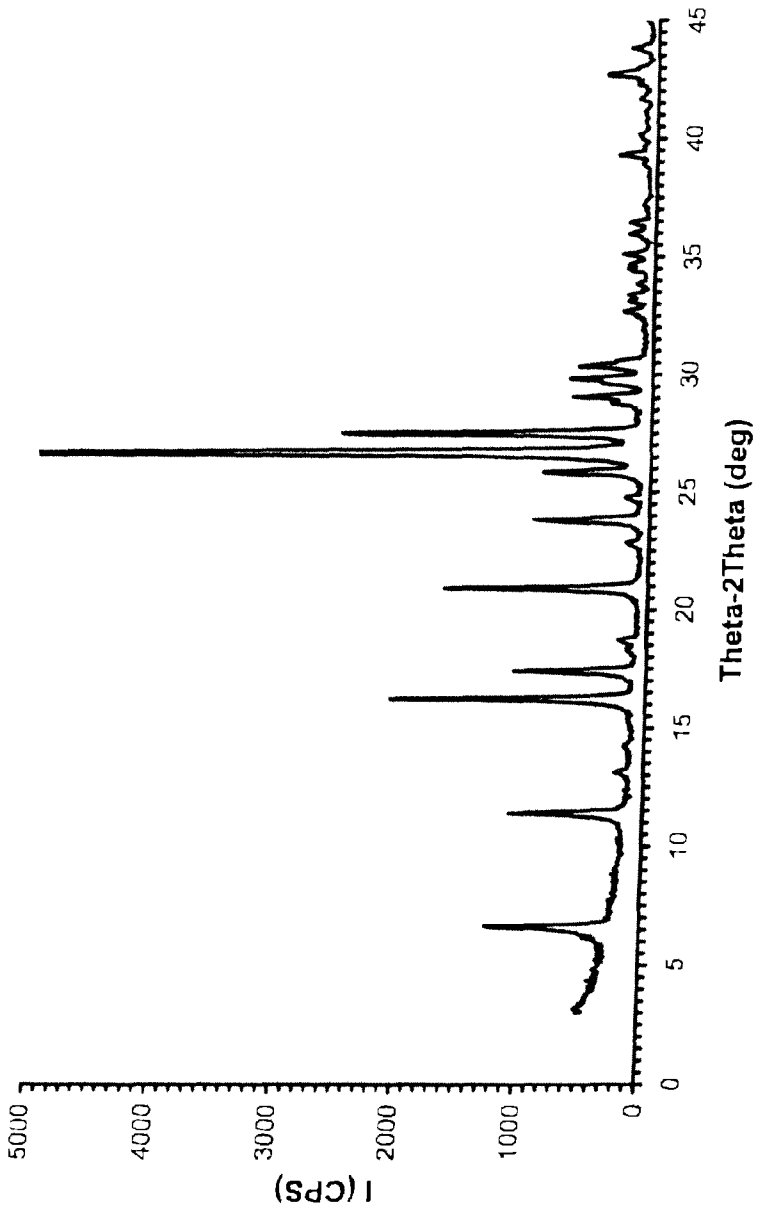
I (CPS)
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 6A

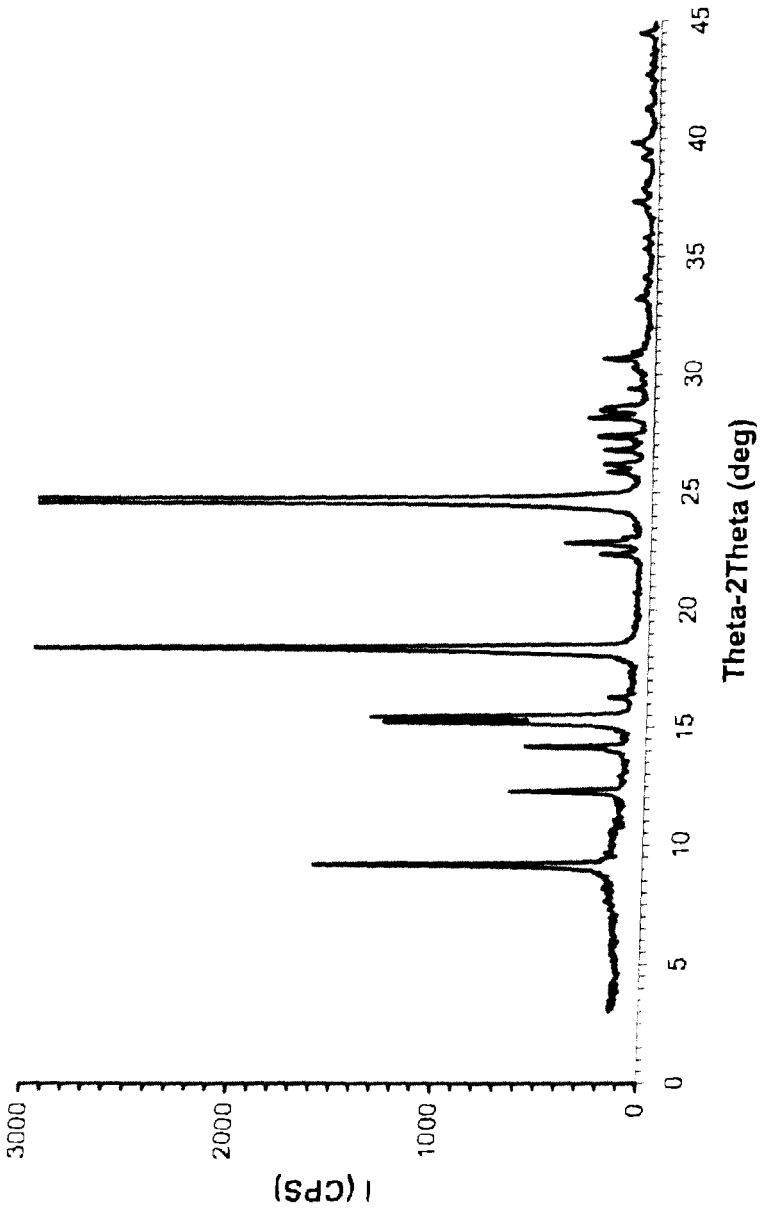

FIG. 6B

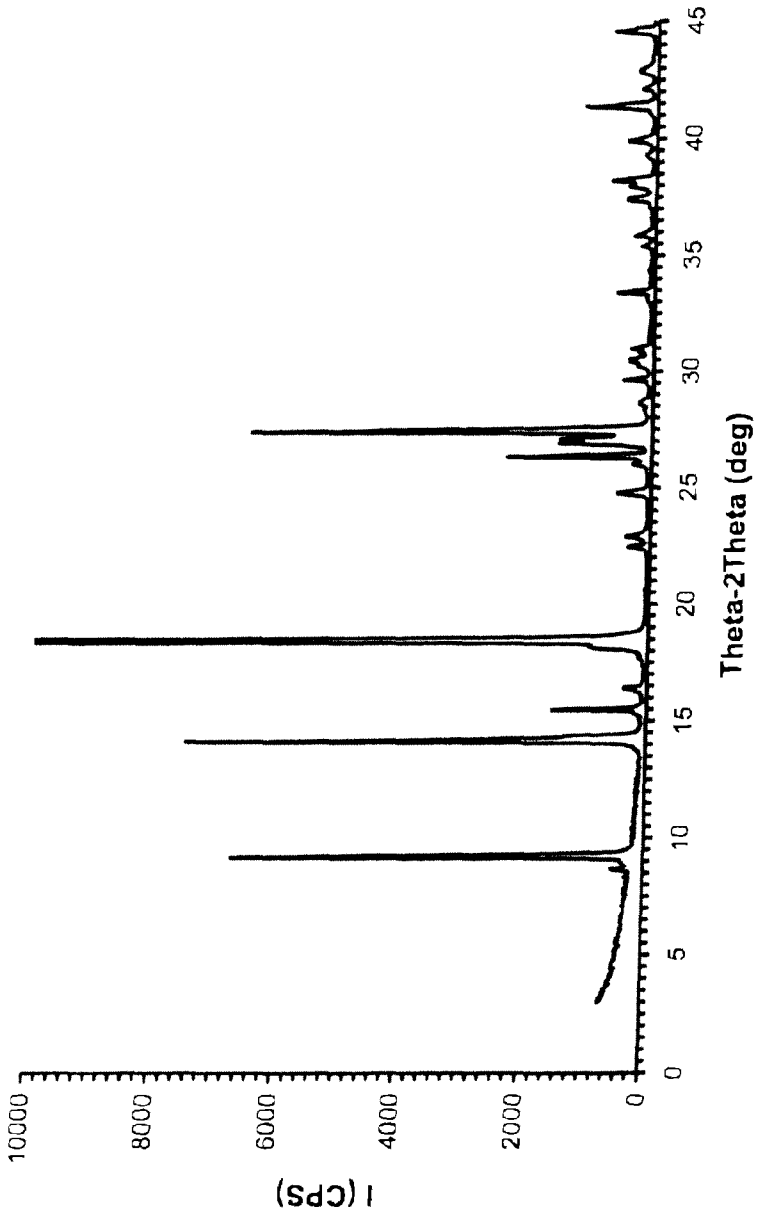
I (CPS)
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 6C

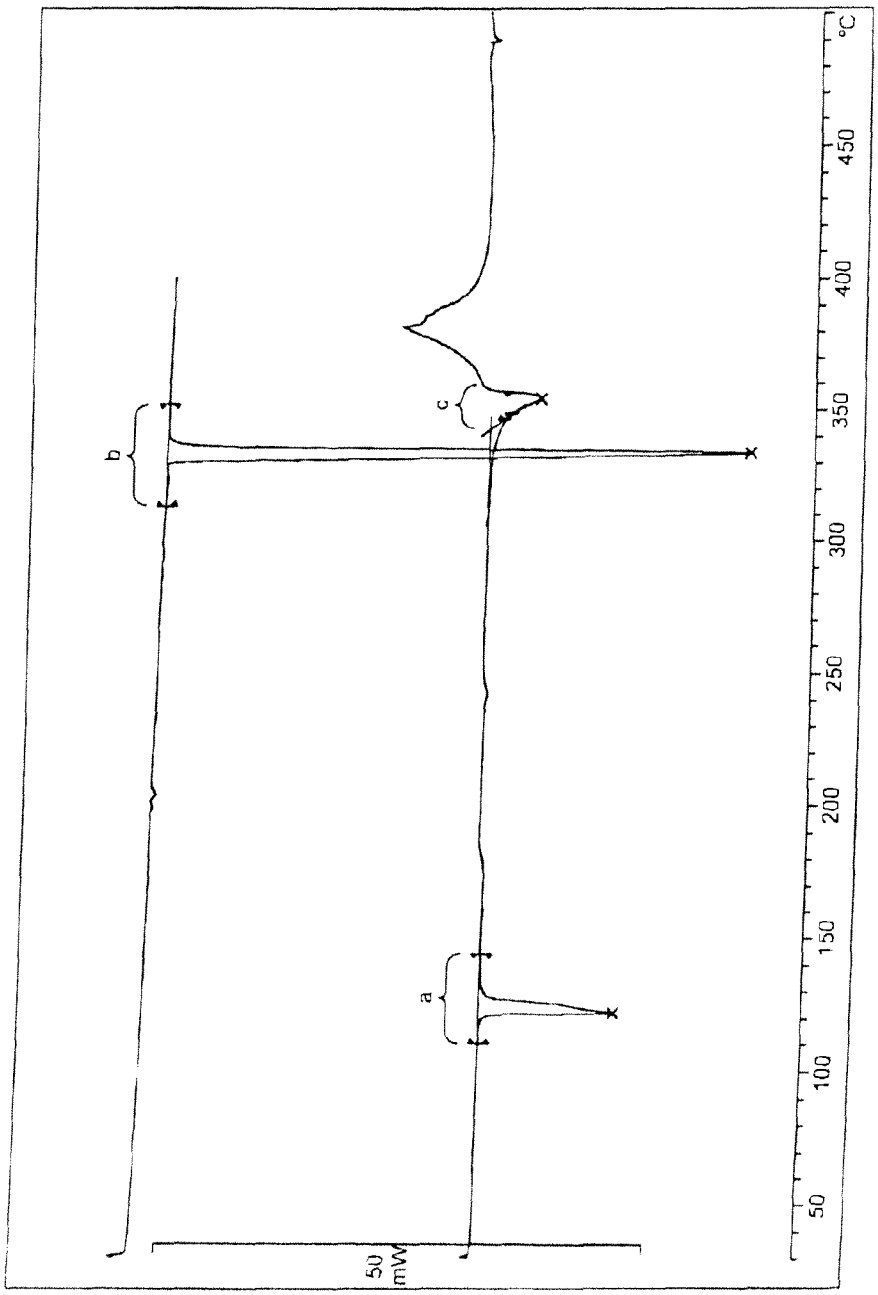

FIG. 7

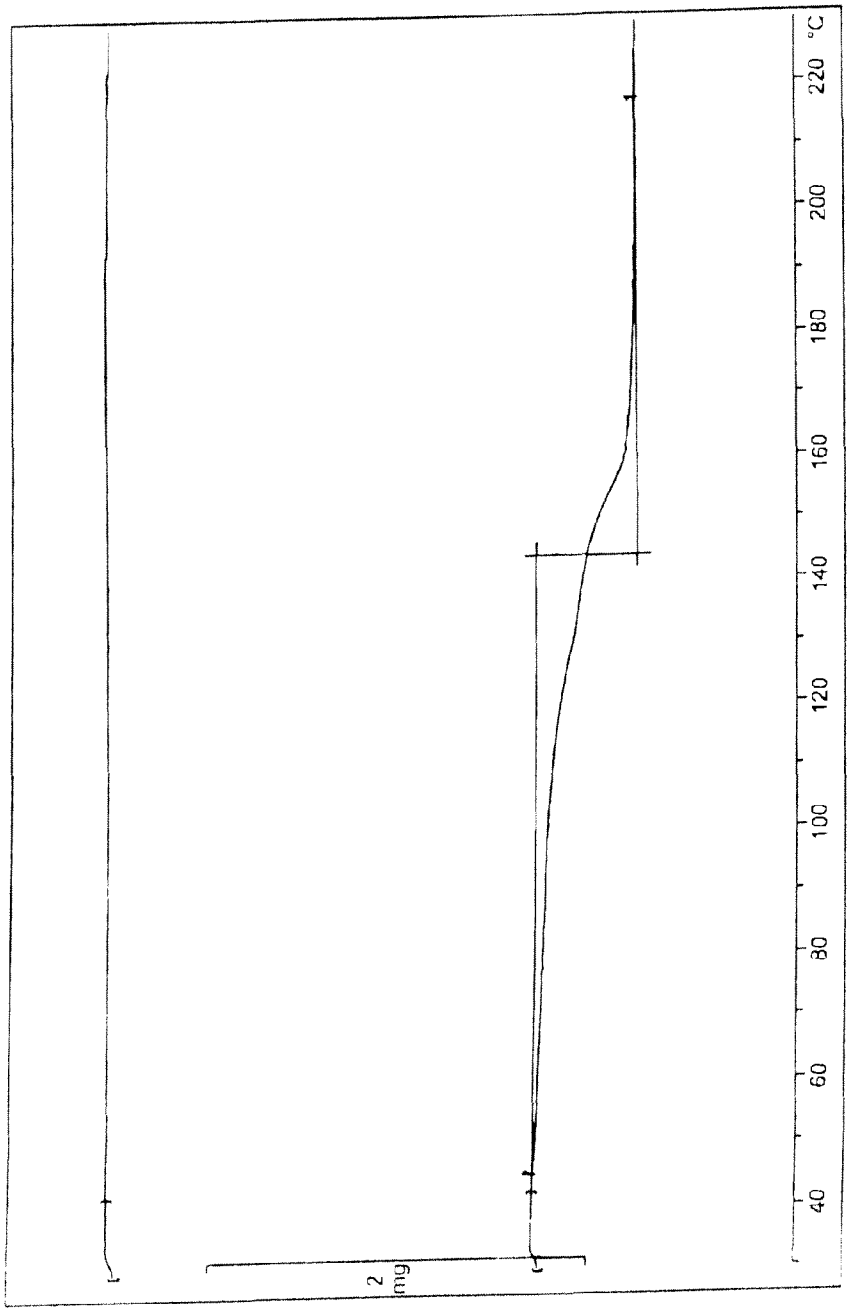

FIG. 8

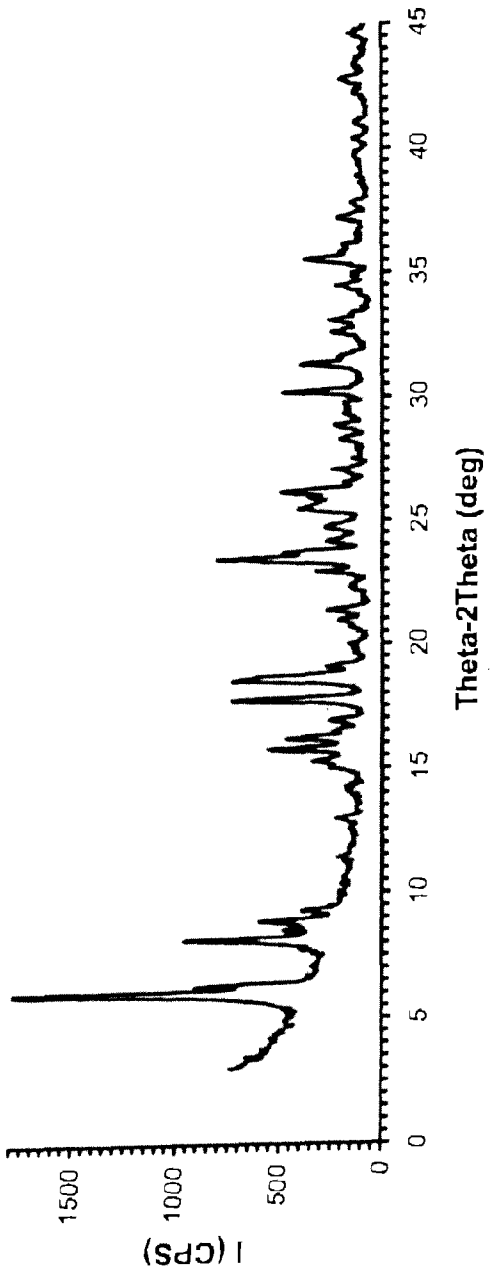

FIG. 9A

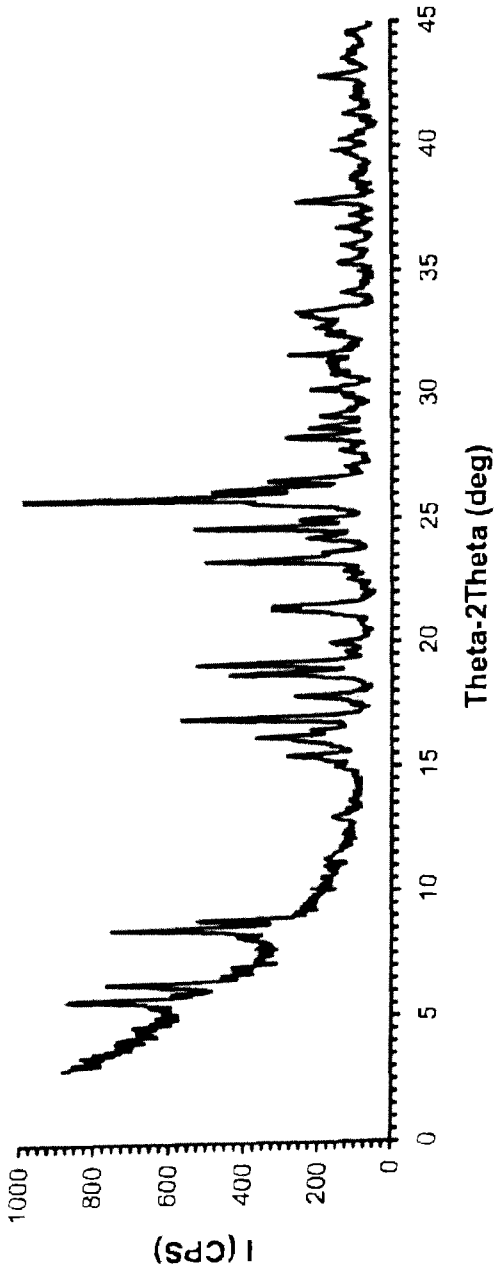
I (CPS)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 9B

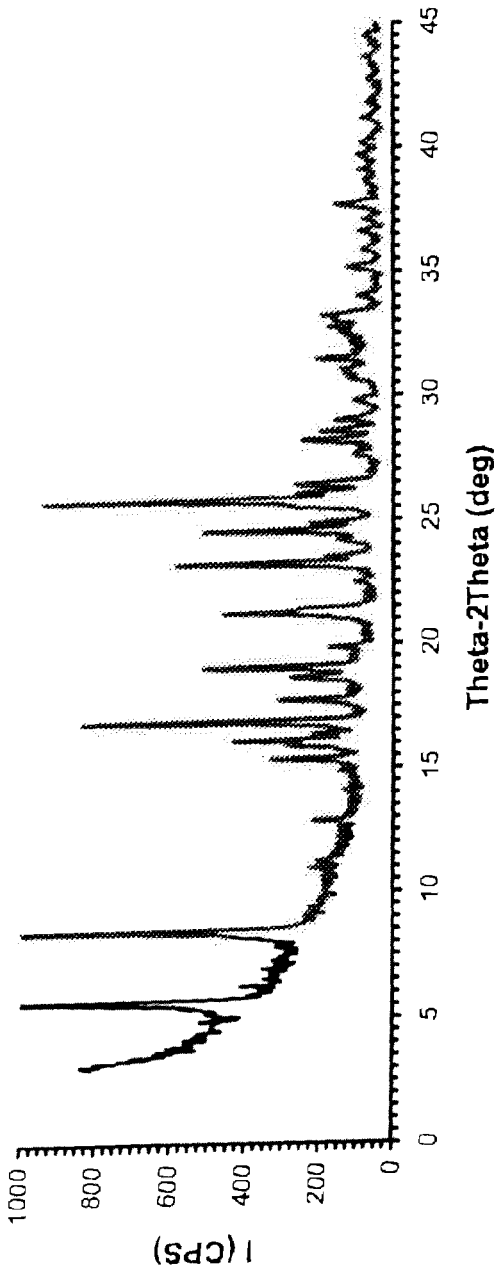
I (CPS)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 9C

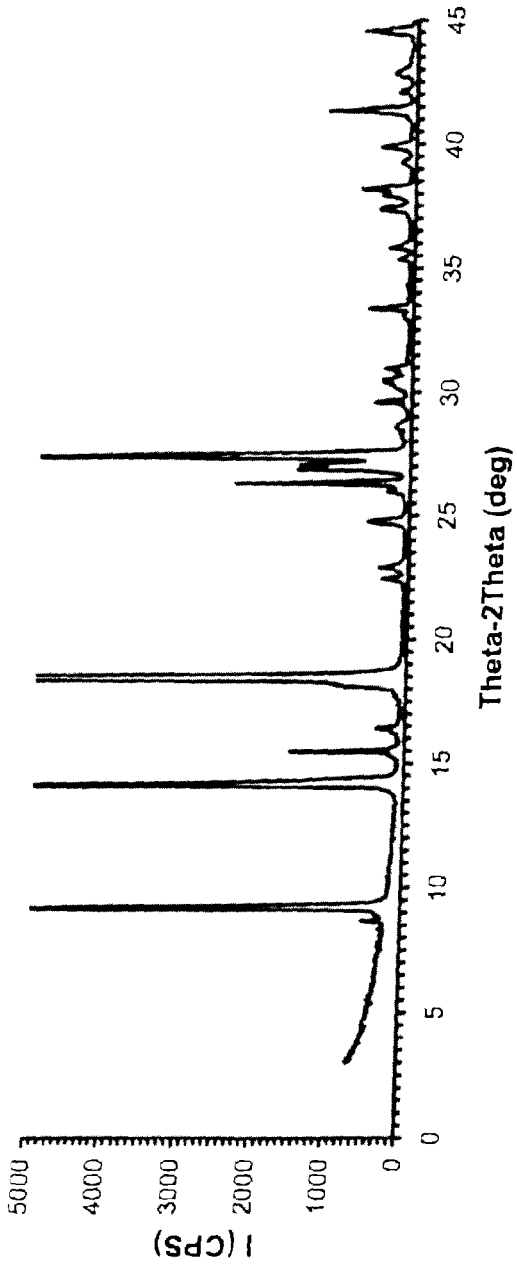
I (CPS)
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 10A

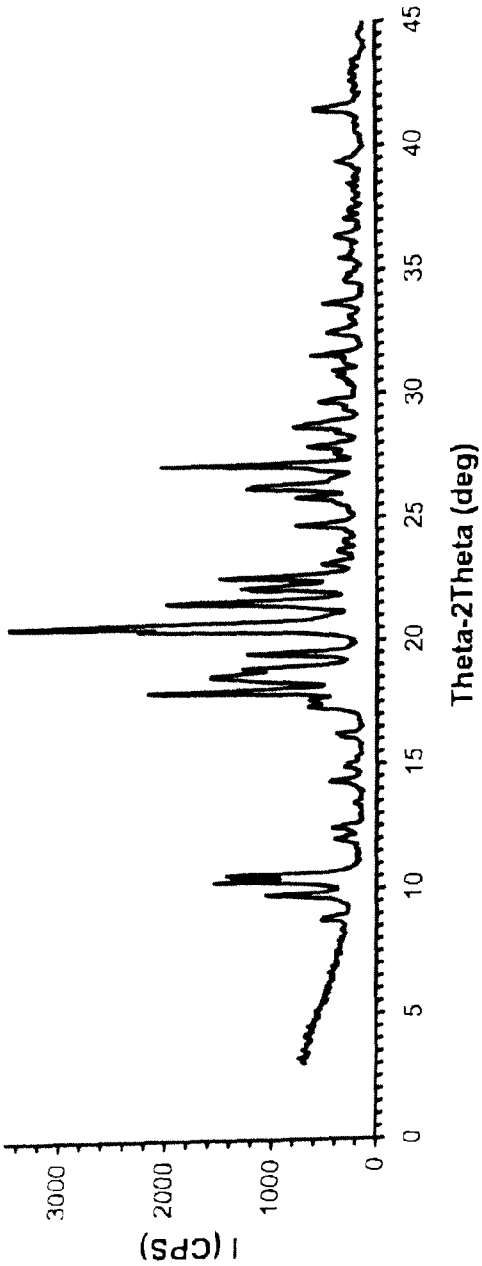

FIG. 10B

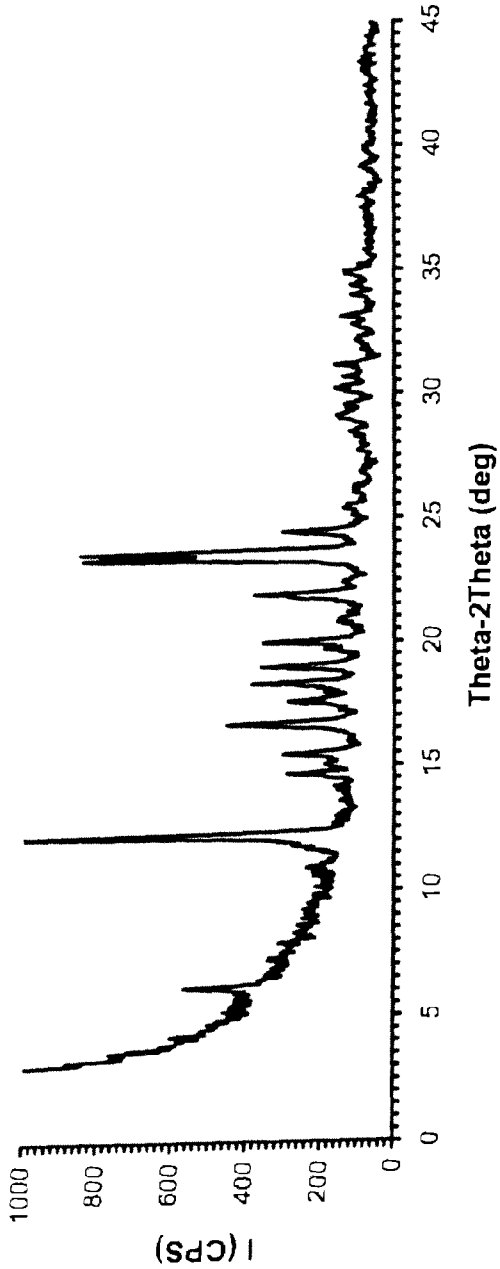
I (CPS)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 10C

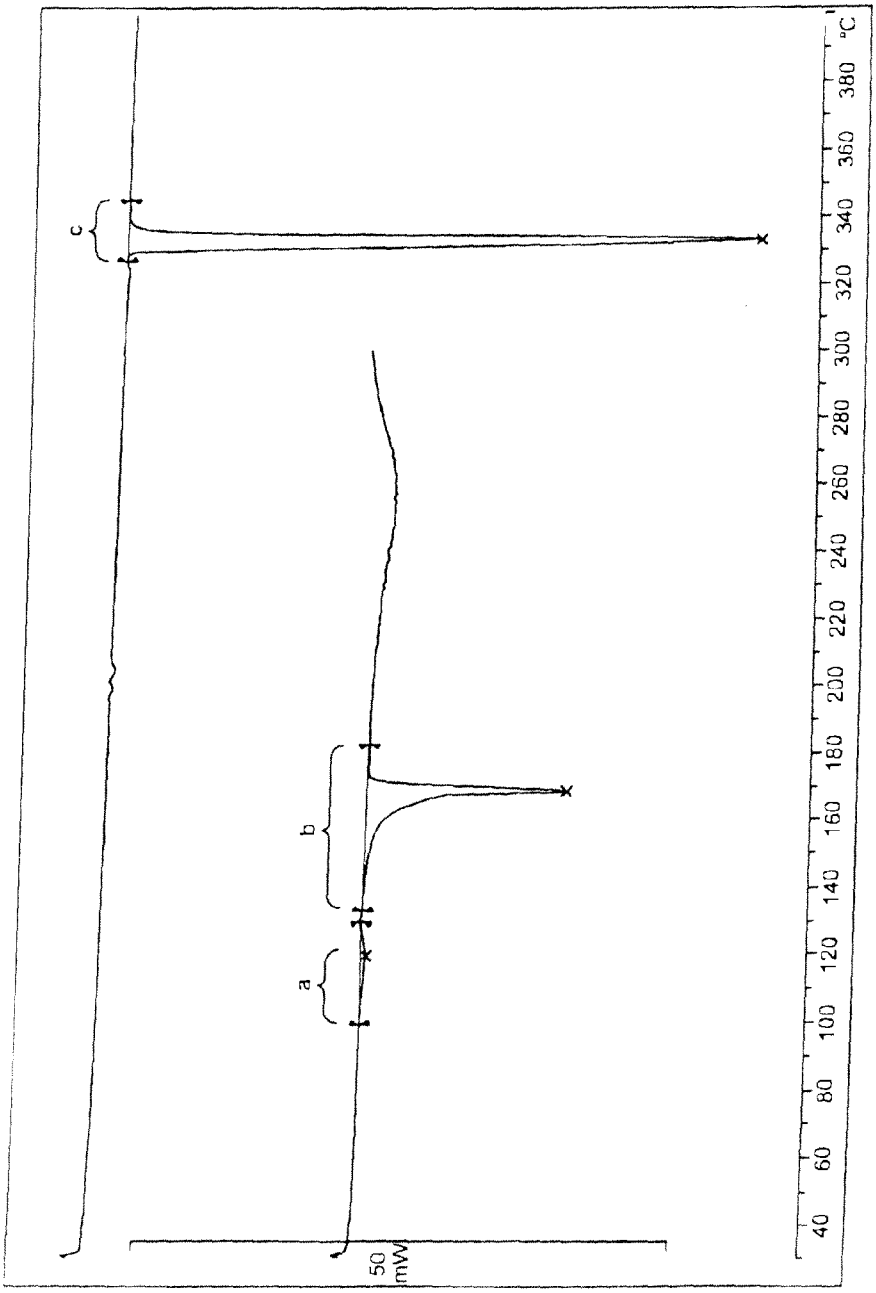
c
b
a
50 mW
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 °C

FIG. 11

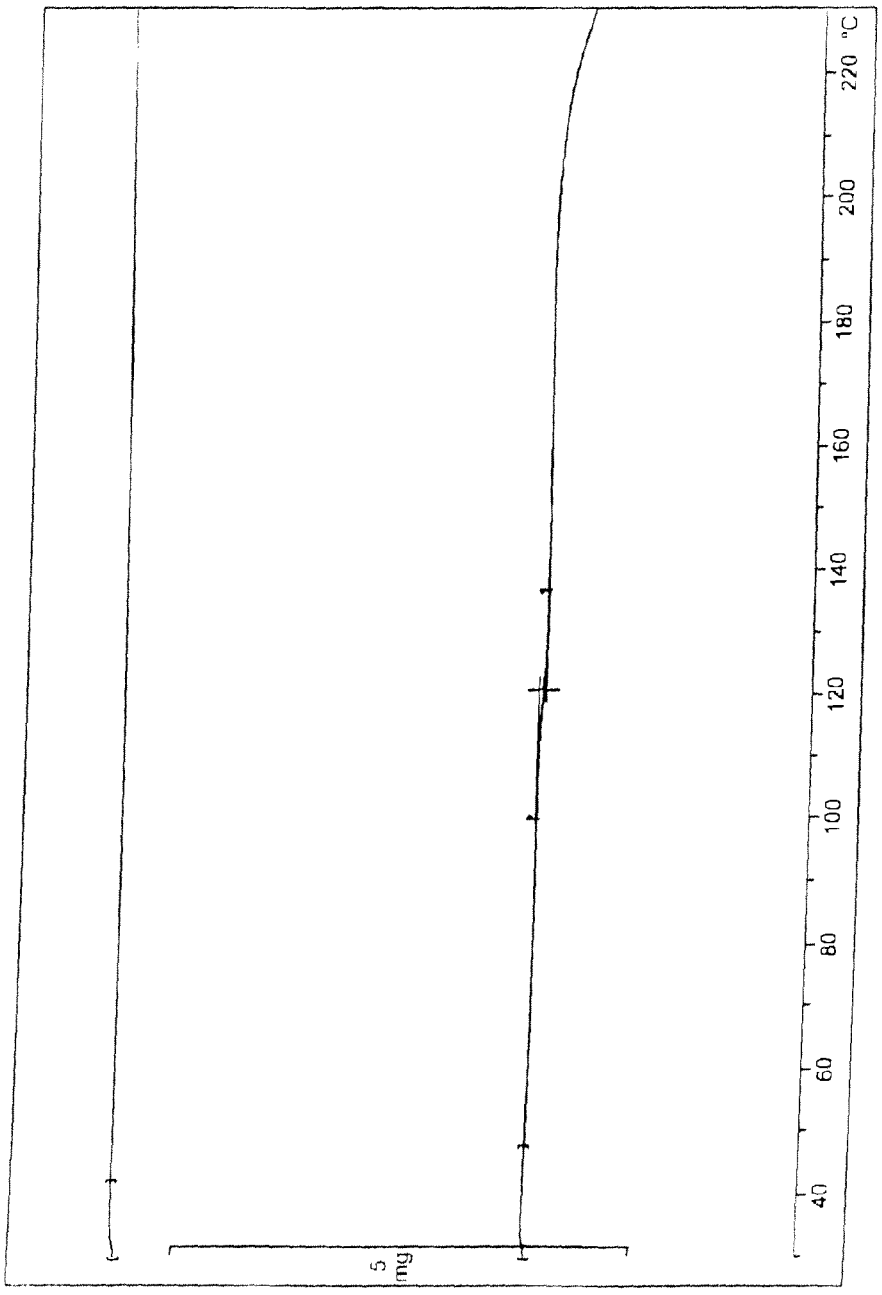

FIG. 12

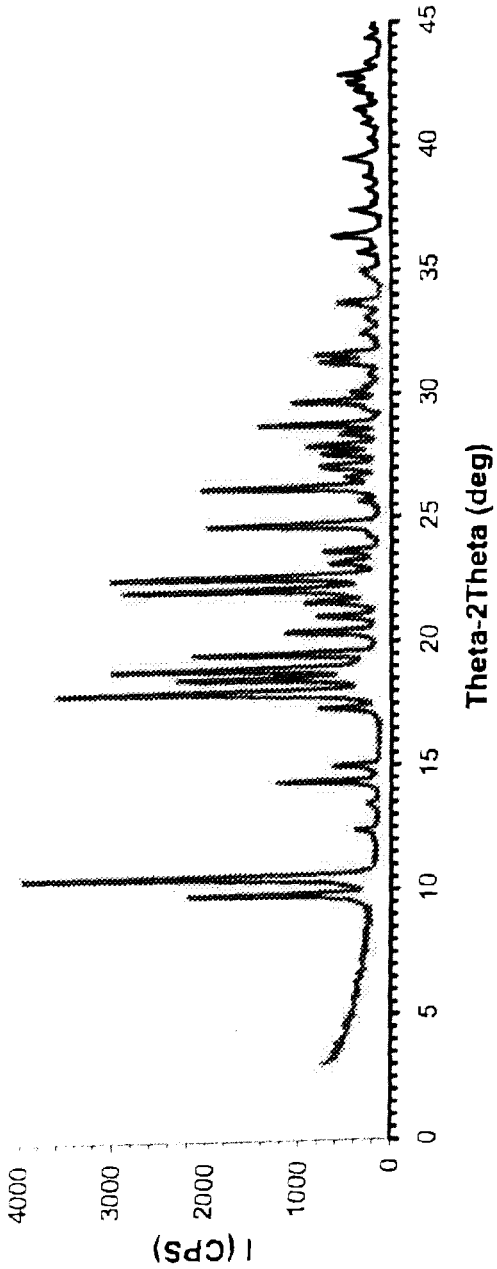
I (CPS)
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 13A

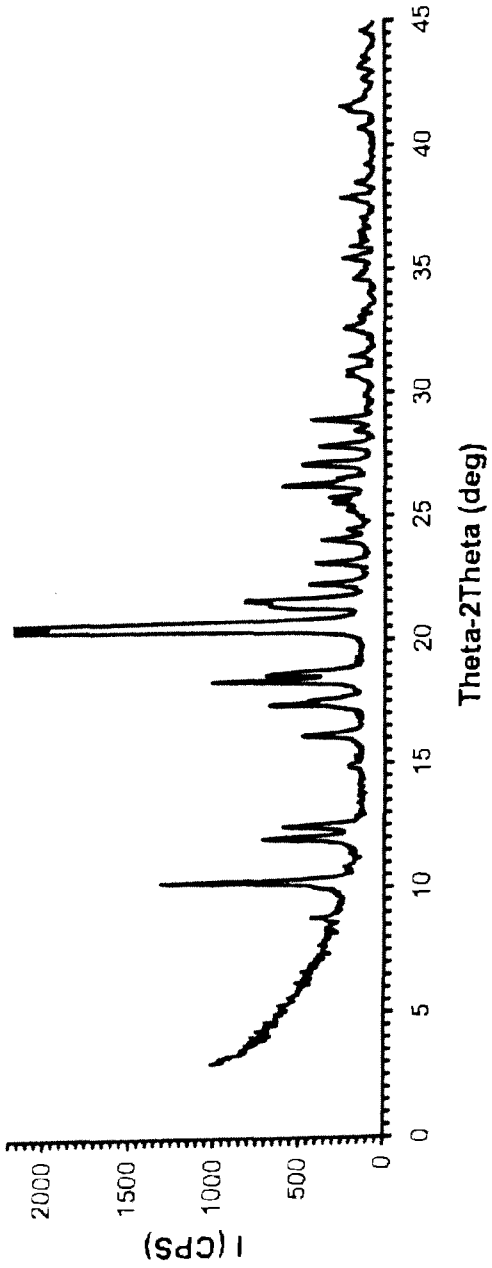
I (CPS)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 13B

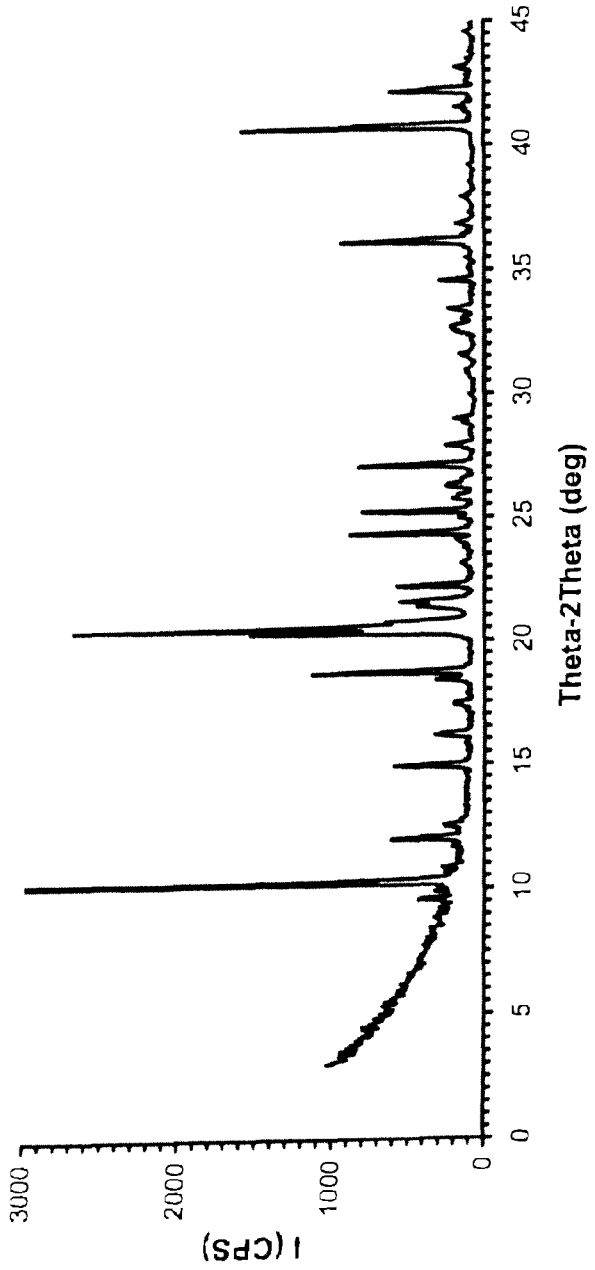

FIG. 13C

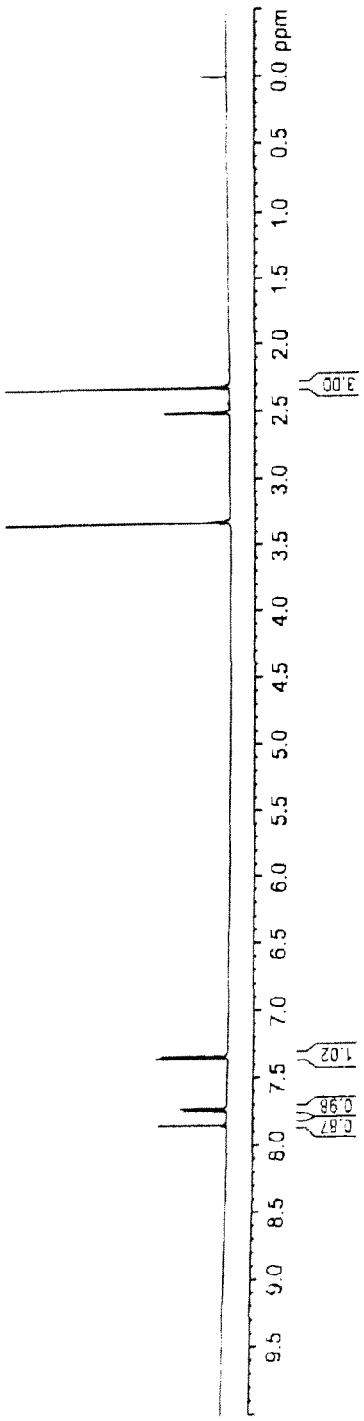
0.0 ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
3.00
1.02
0.98
0.87

FIG. 14A

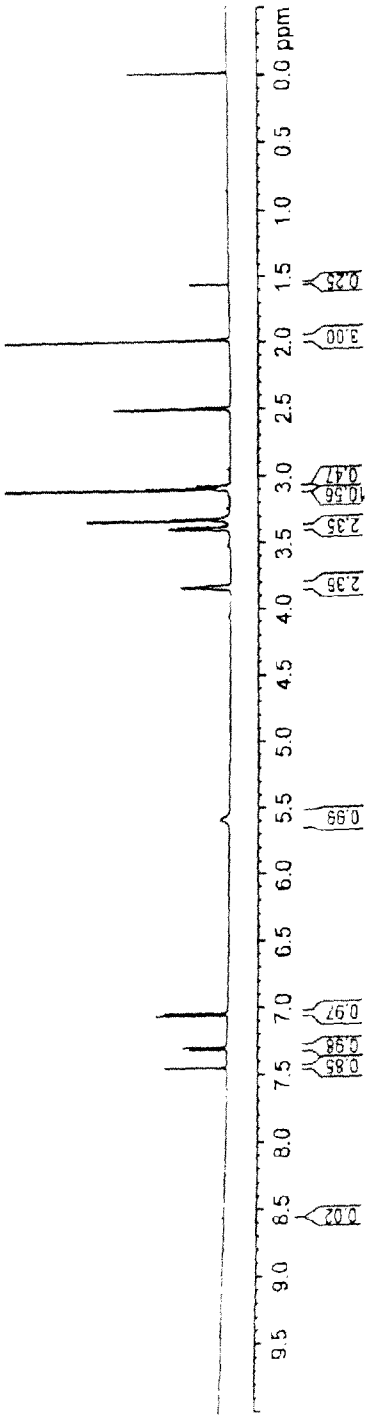
0.0 ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
0.25
3.00
0.47
10.56
2.35
2.35
0.99
0.97
0.98
0.85
0.02

FIG. 14B

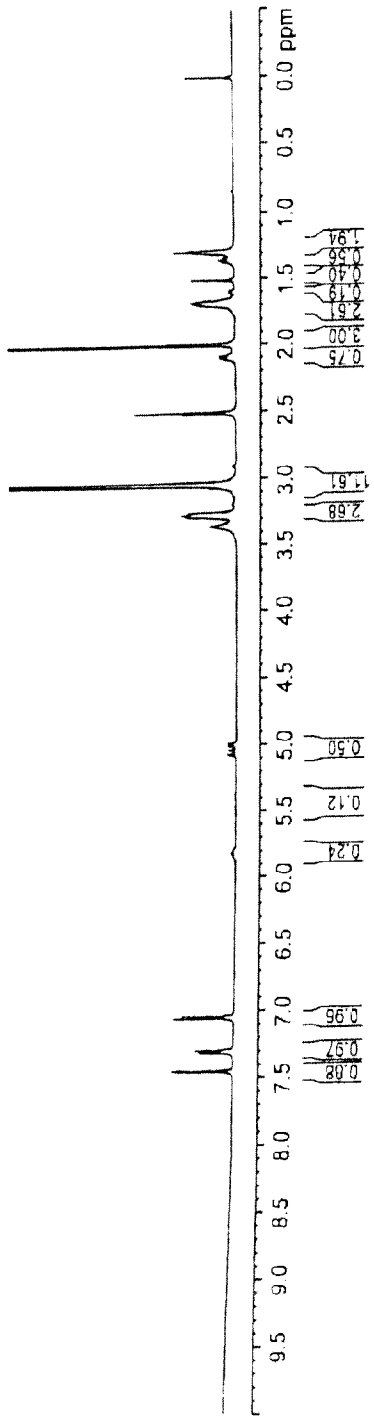
0.0 ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
0.08
0.97
0.96
0.24
0.12
0.50
2.68
11.61
0.75
3.00
2.61
0.19
0.49
0.56
1.94

FIG. 14C

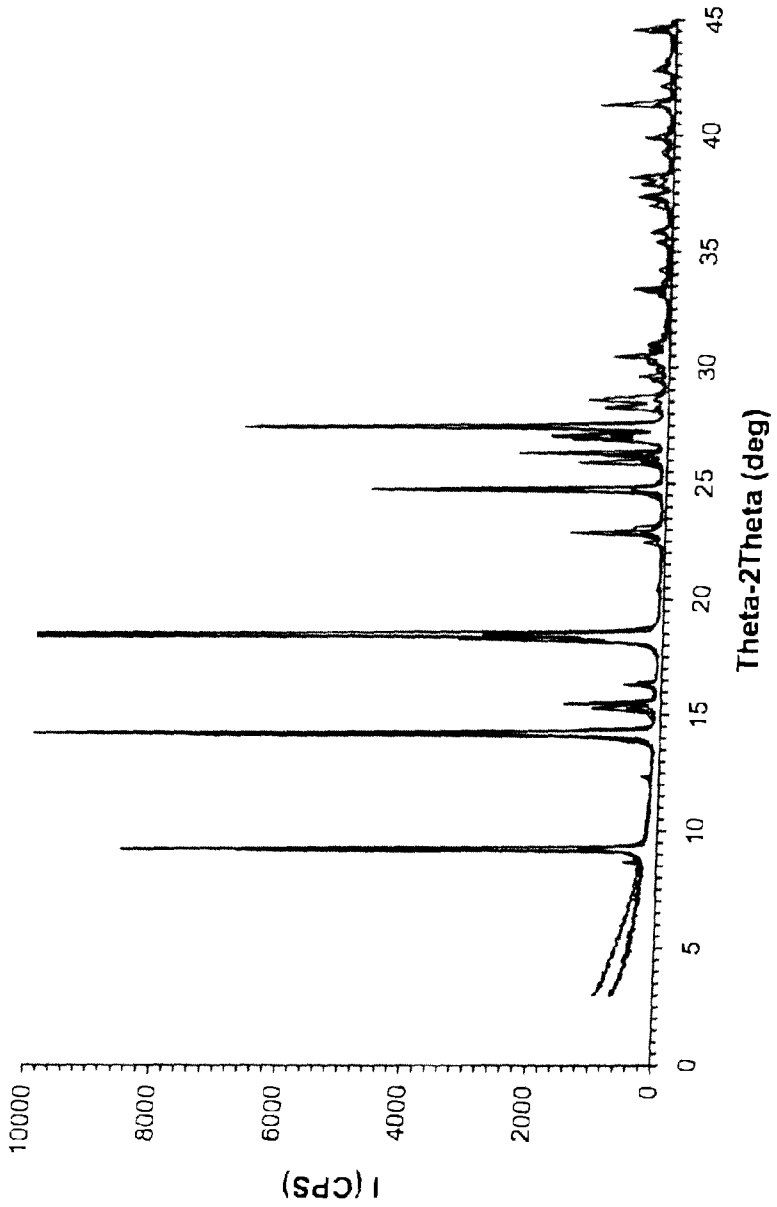
I (CPS)
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 15A

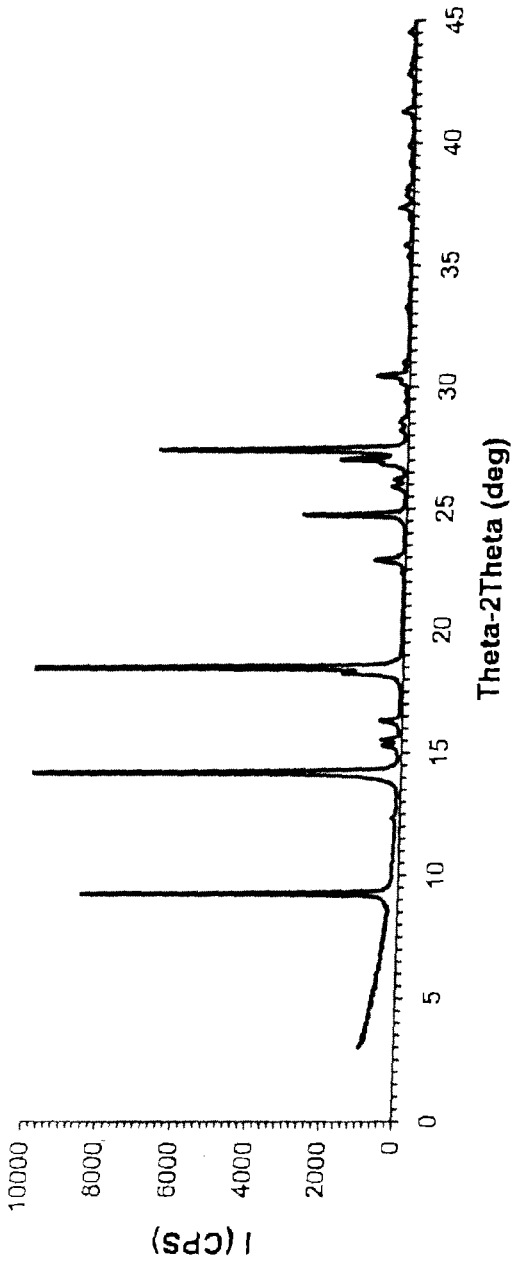
I (CPS)
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 15B

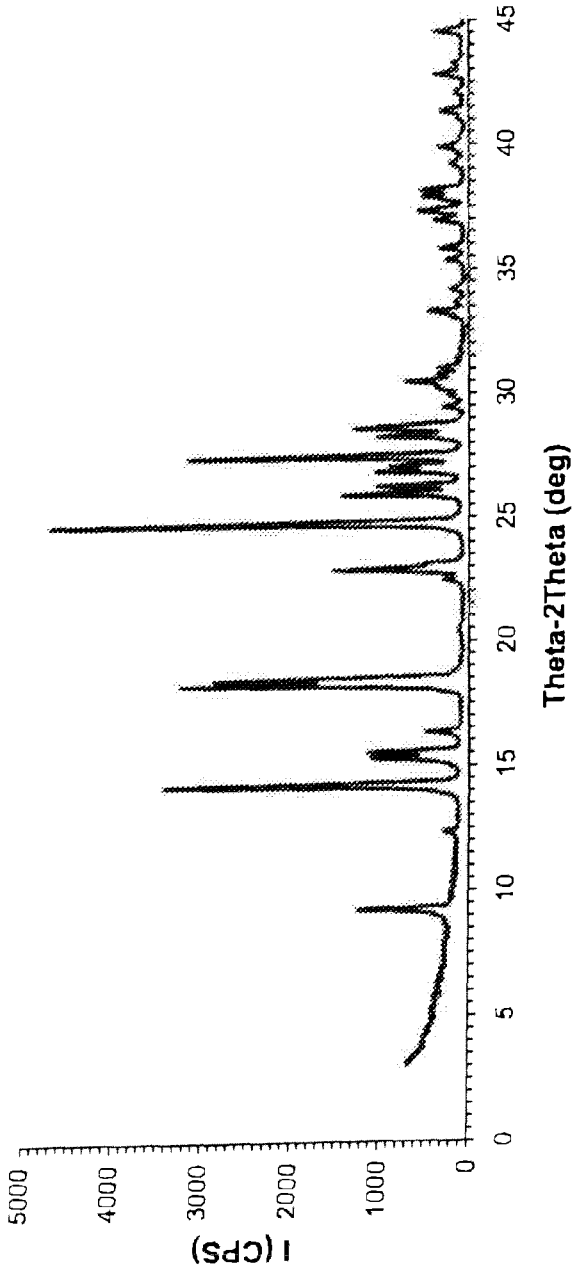
I (CPS)
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 15C

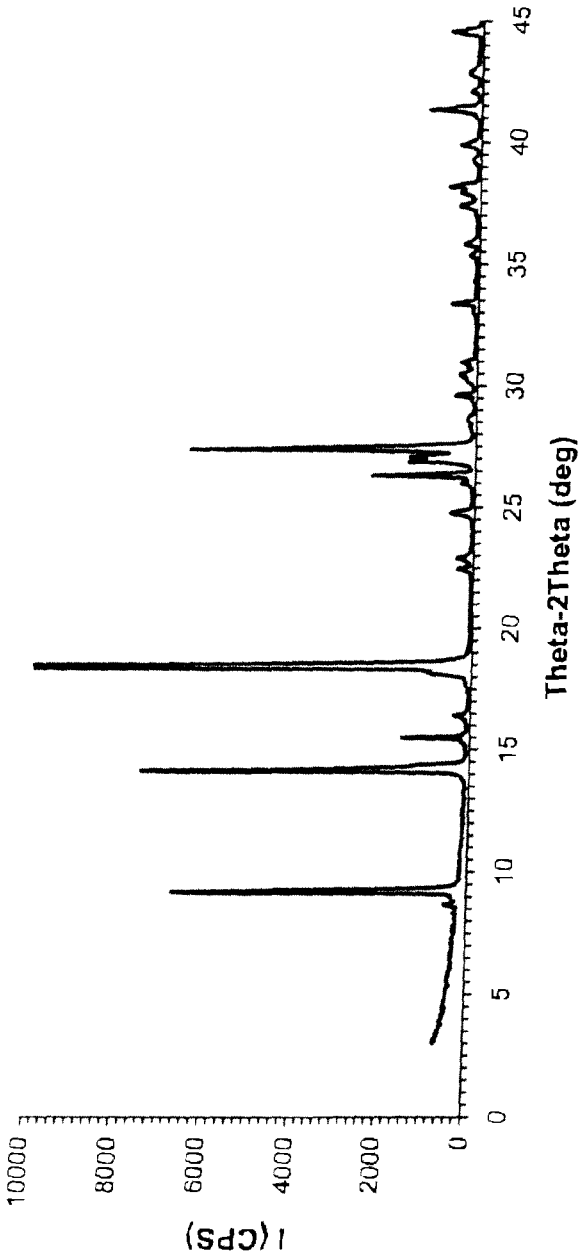
I (CPS)
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 15D

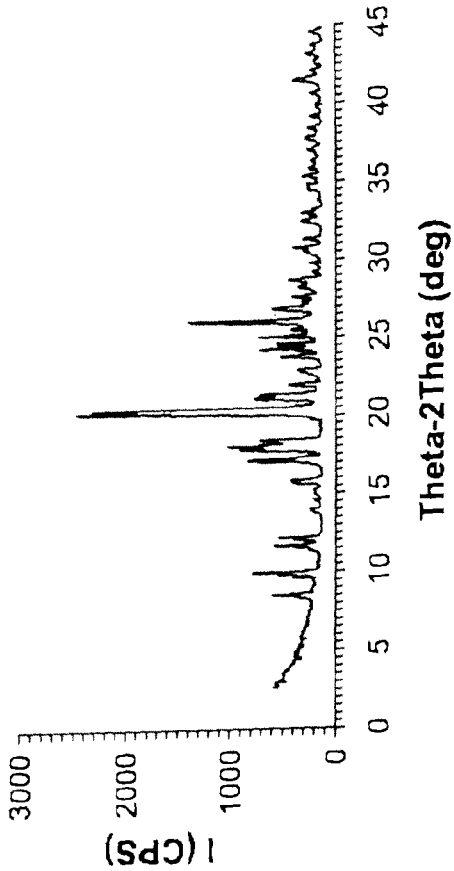

FIG. 16A

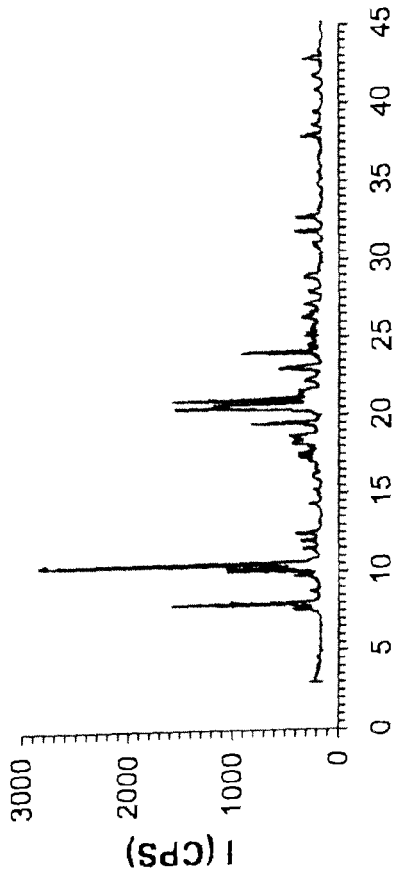

FIG. 16B

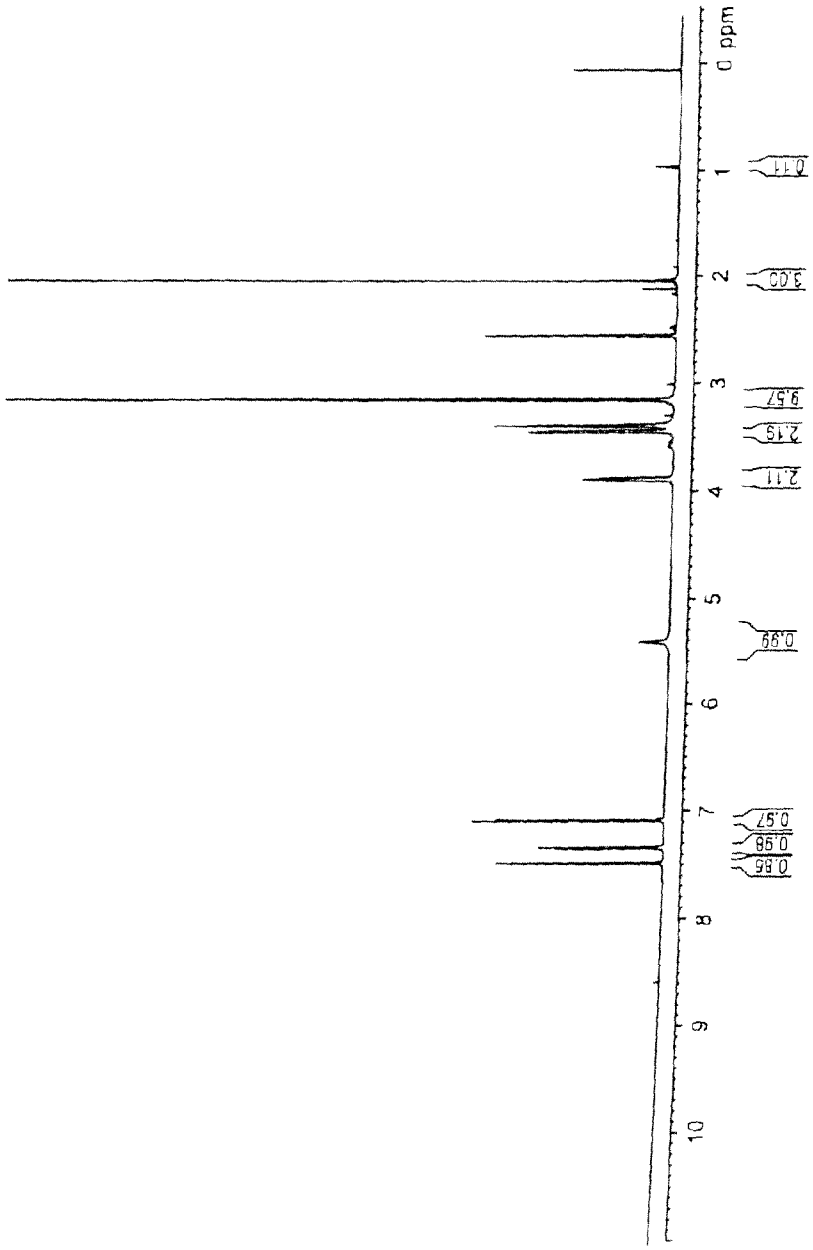

FIG. 17A

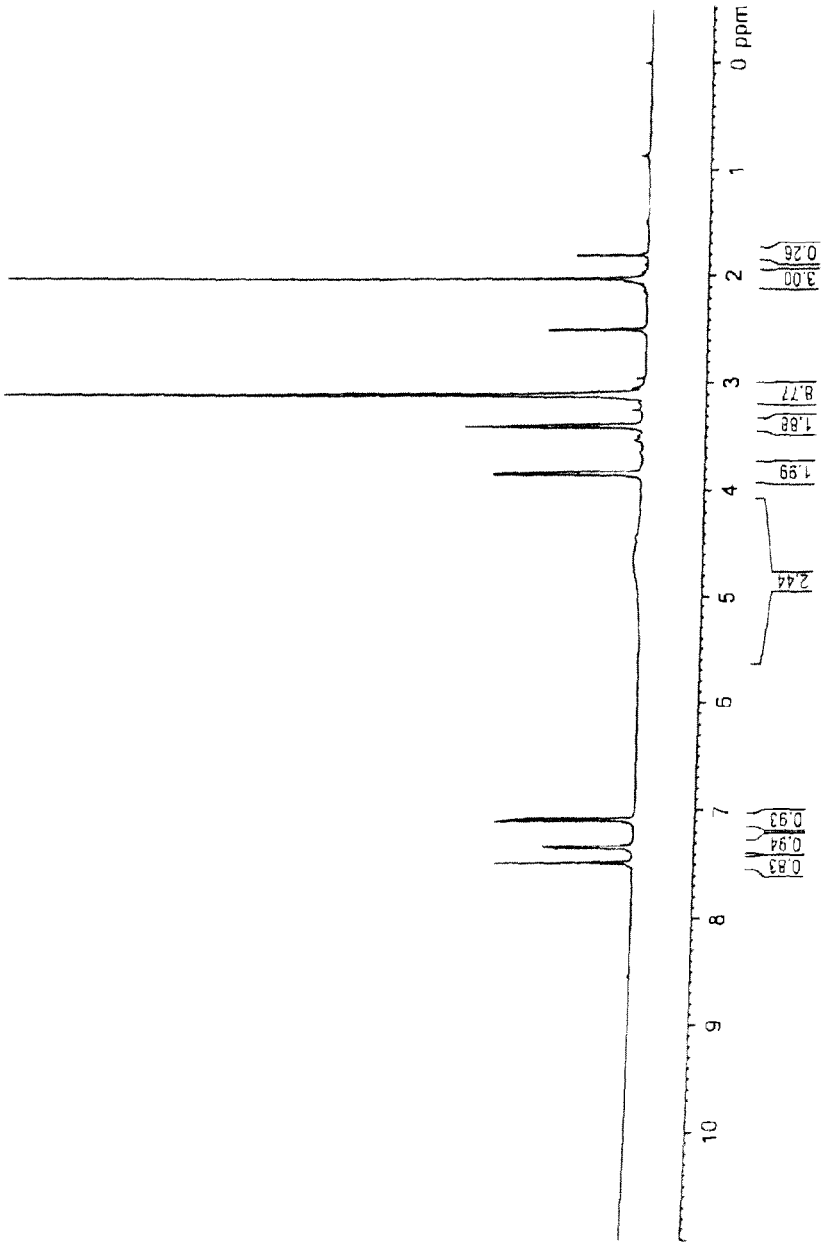

FIG. 17B

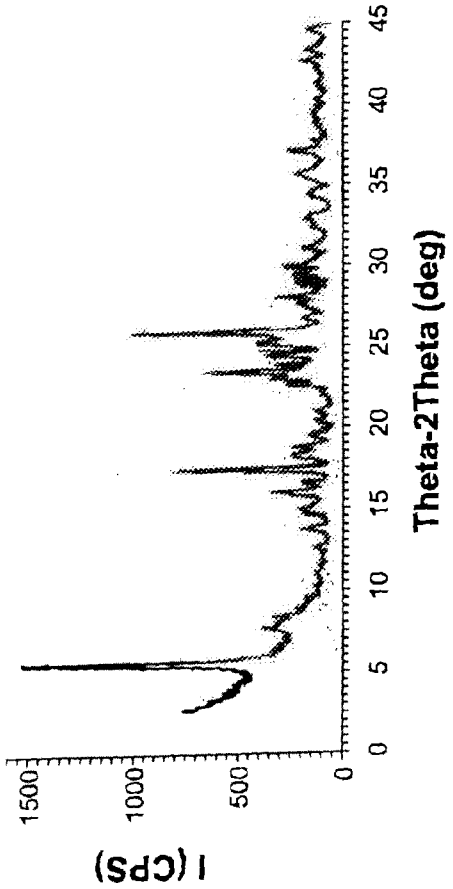

FIG. 18A

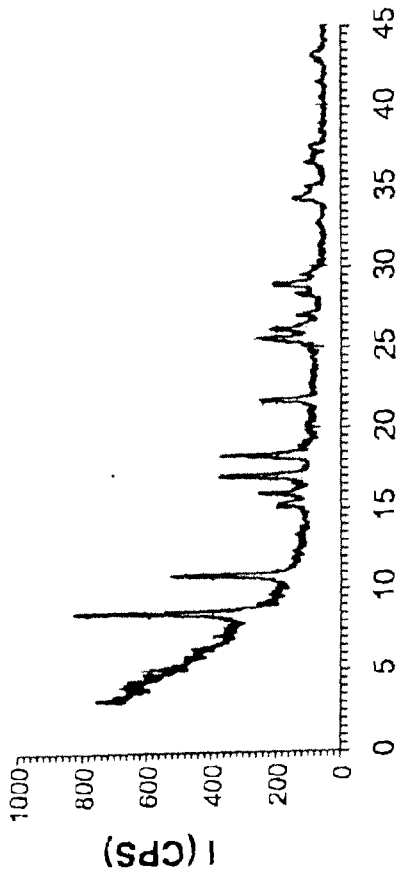
I (CPS)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 18B

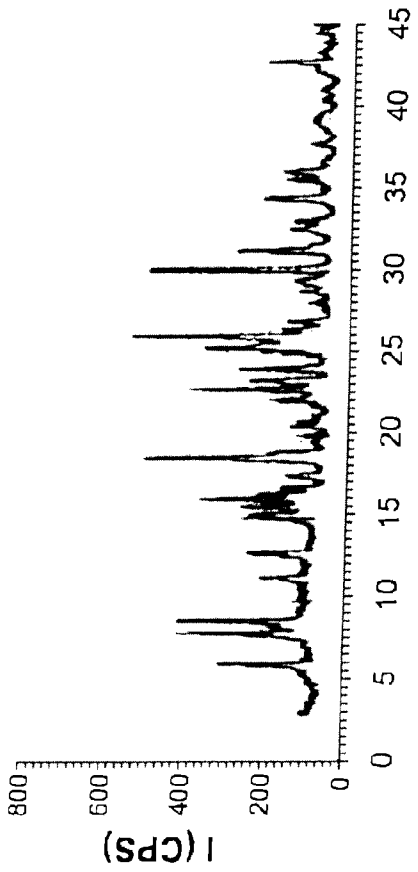

FIG. 18C

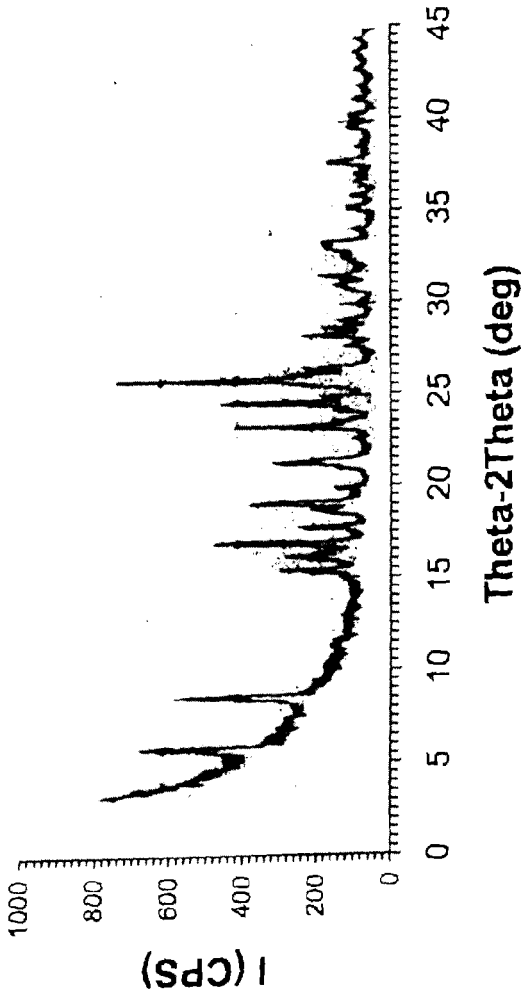
I (CPS)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 19A

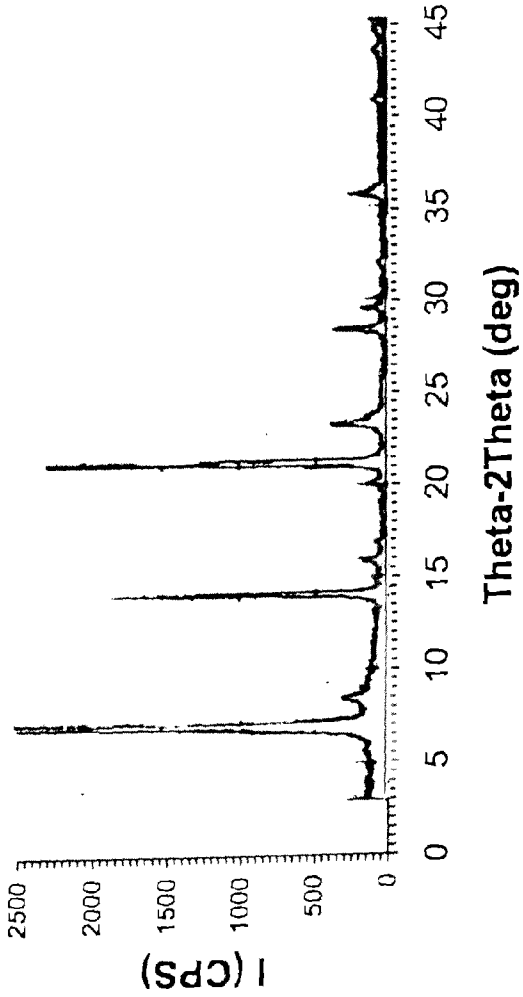
I (CPS)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 19B

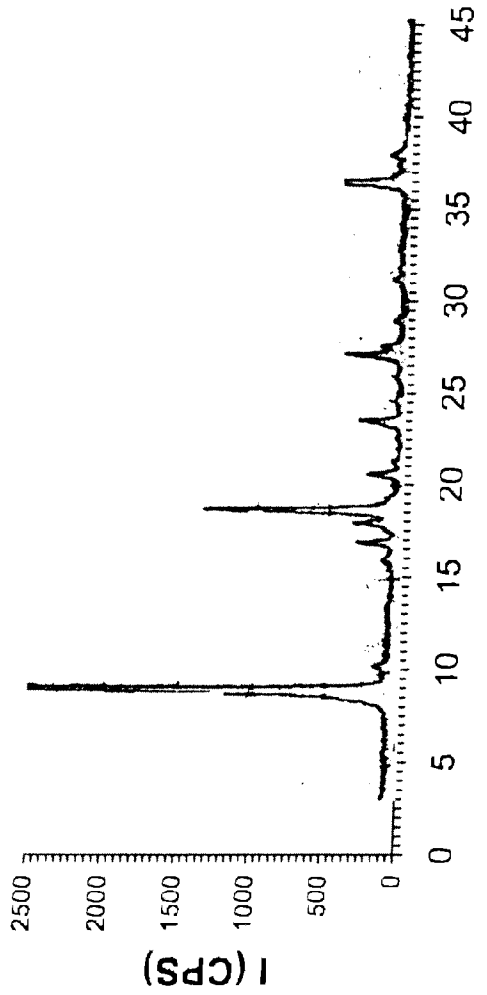
I (CPS)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
45
Theta-2Theta (deg)

FIG. 19C

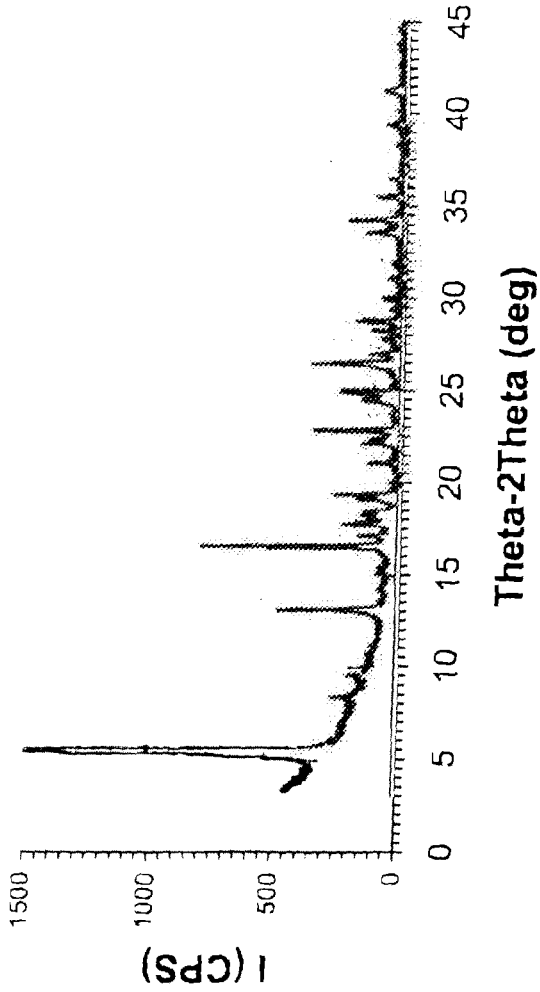

FIG. 19D

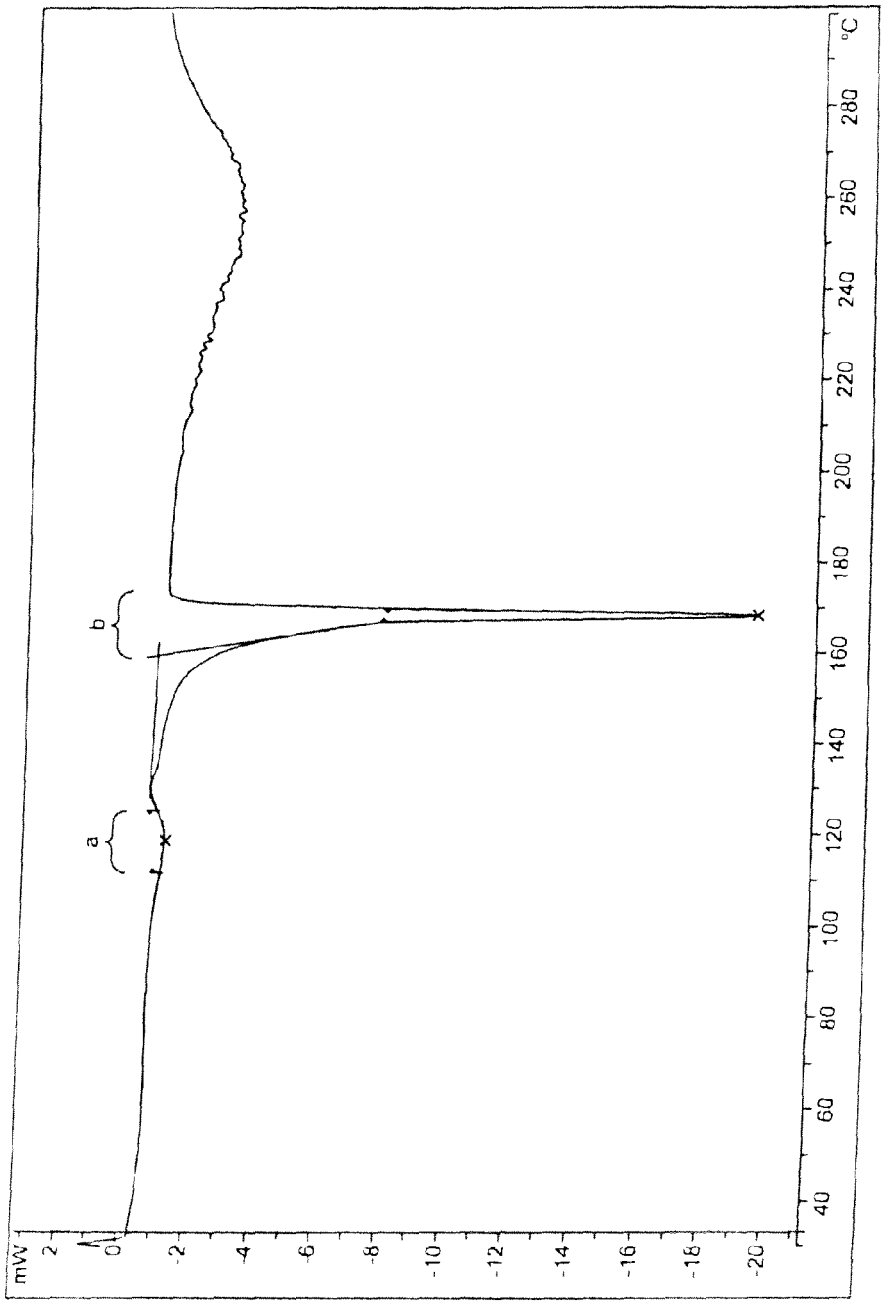
mW
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
a
b
40
60
80
100
120
140
160
180
200
220
240
260
280
°C

FIG. 20

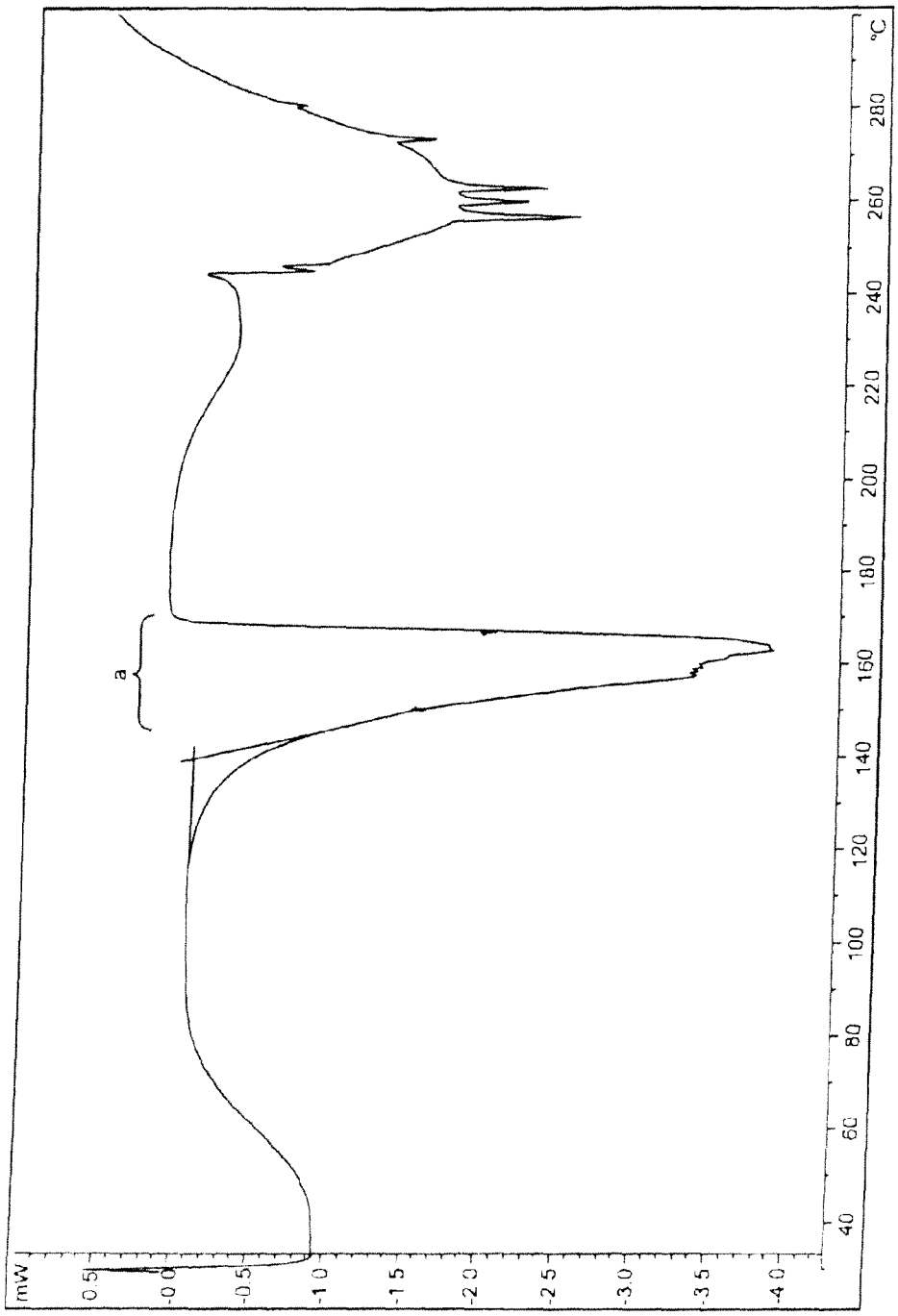
mW
0.5
-0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
a
40
60
80
100
120
140
160
180
200
220
240
260
280
°C

FIG. 21

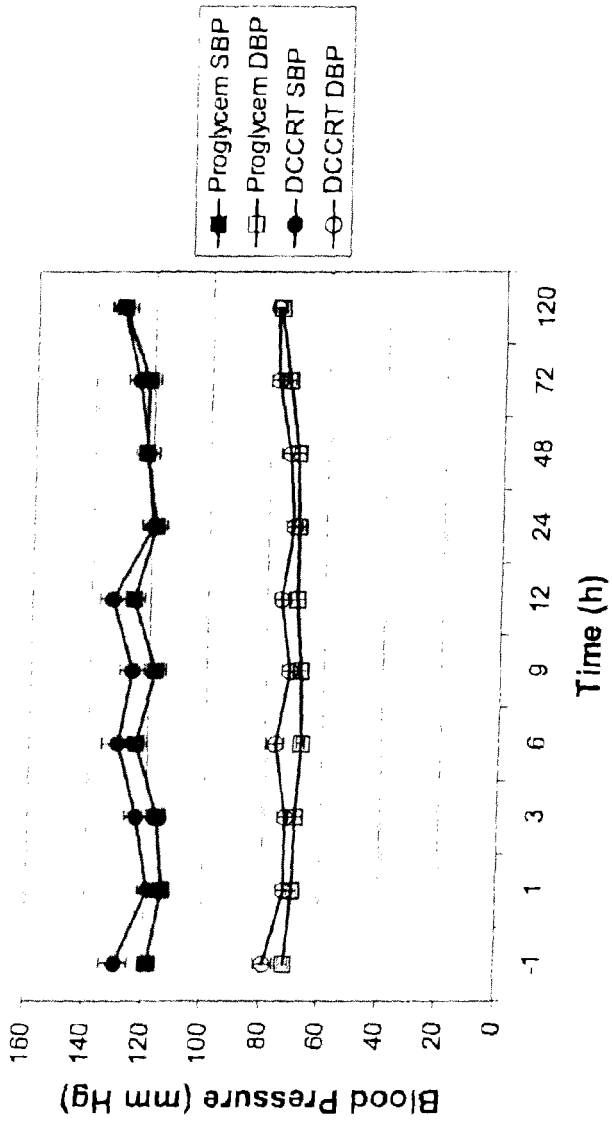
Proglycem SBP
Proglycem DBP
DCCRT SBP
DCCRT DBP
Blood Pressure (mm Hg)
160
140
120
100
80
60
40
20
0
-1
1
3
6
9
12
24
48
72
120
Time (h)

FIG. 22

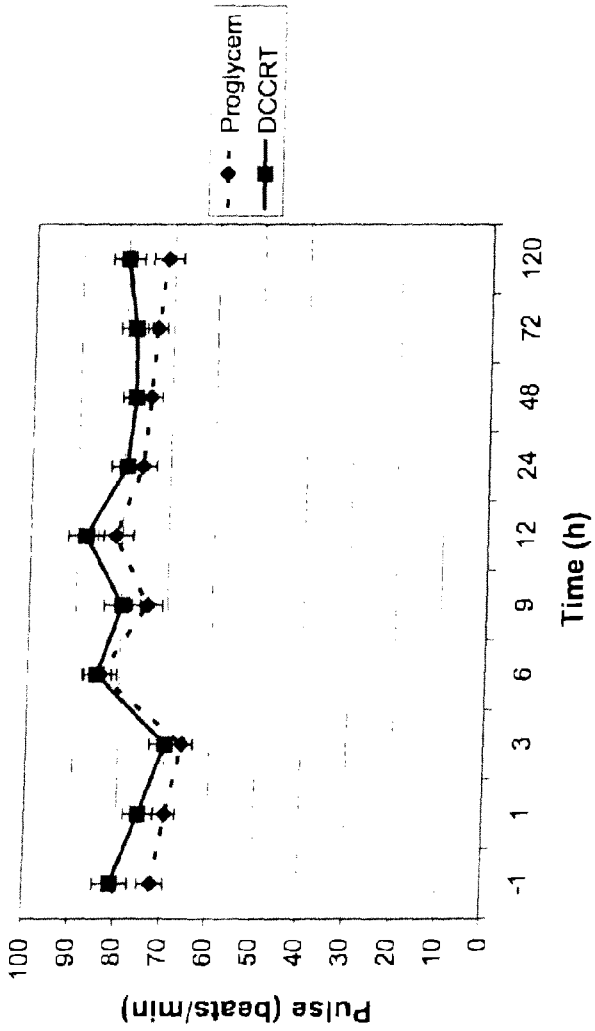
Proglycem
DCCRT
Pulse (beats/min)
100
90
80
70
60
50
40
30
20
10
0
-1
1
3
6
9
12
24
48
72
120
Time (h)

FIG. 23

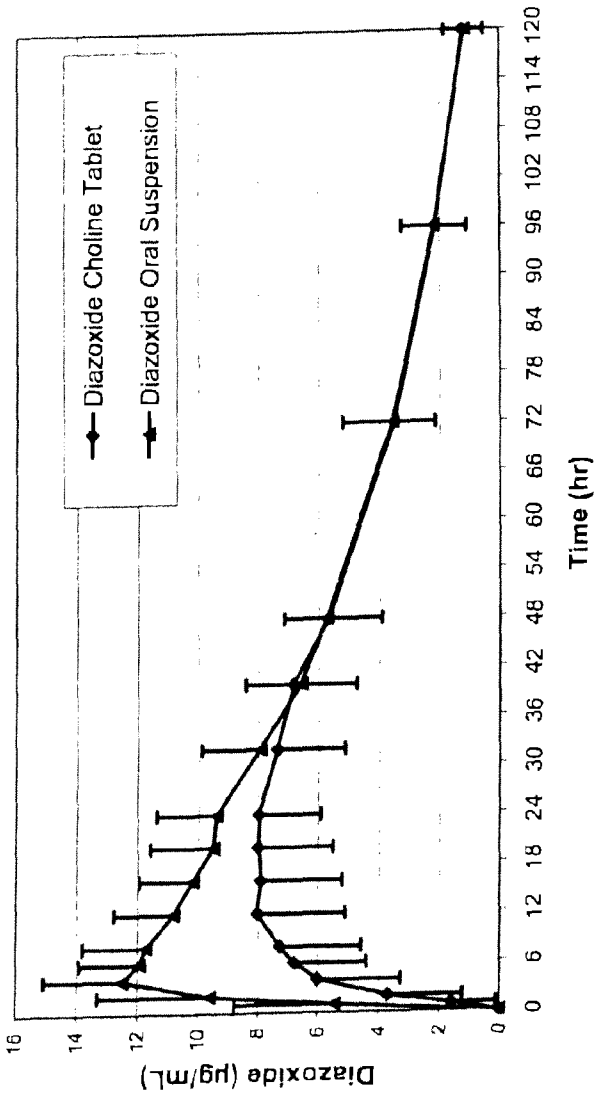
Diazoxide Choline Tablet
Diazoxide Oral Suspension
Diazoxide (µg/mL)
Time (hr)
0
2
4
6
8
10
12
14
16
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
108
114
120

FIG. 24

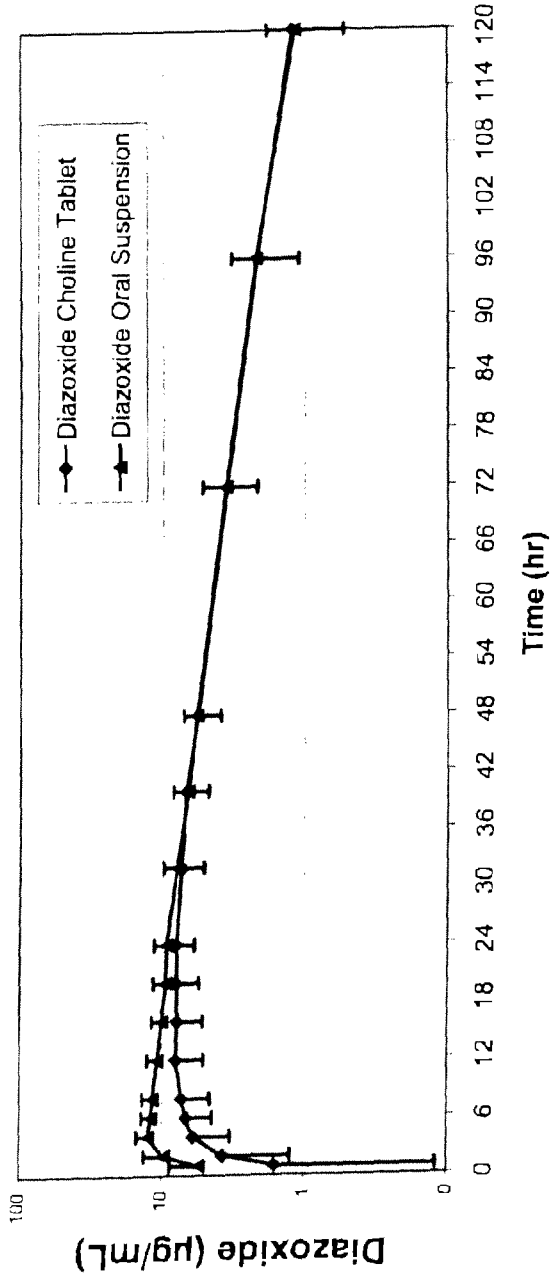
Diazoxide Choline Tablet
Diazoxide Oral Suspension
Diazoxide (µg/mL)
100
10
1
0
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102 108 114 120
Time (hr)

FIG. 25

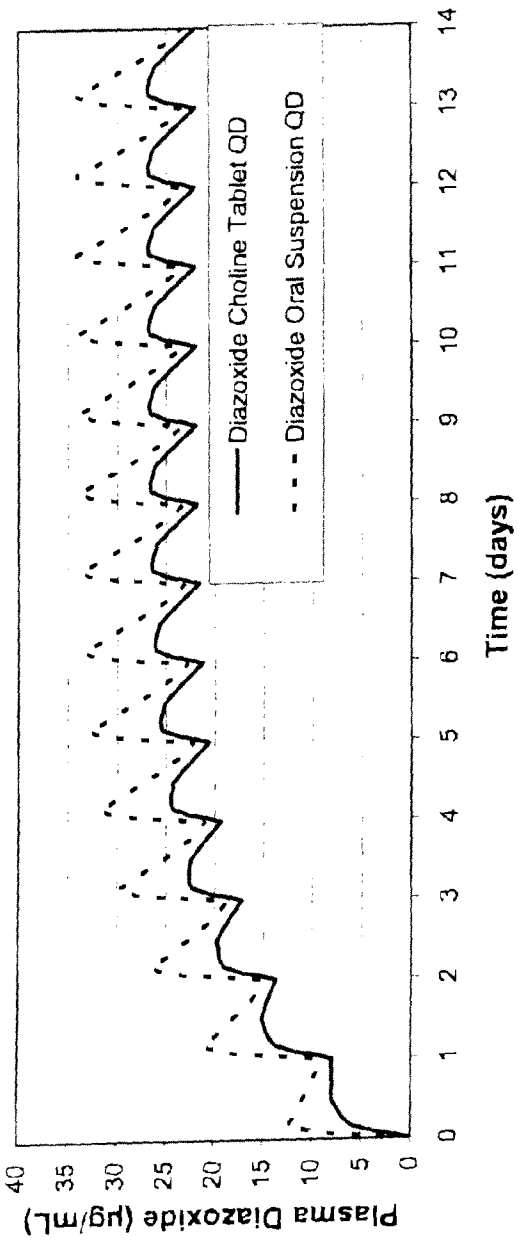

FIG. 26

APOTEX_EX1009_PGR2026-00034_Page 293

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/68936

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/54 (2008.04)
USPC - 514/223.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 514/223.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic Databases Searched: USPTO WEST (PGPUB, EPAB, JPAB, USPT), Google Patent, Google Scholar. Search Terms Used: benzothiadiazine, thyroid, pancreatitis or metab$, alkali metal, diazoxide choline, pharmac$ salt, channel opener

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2006/026469 A2 (Cowen et al.) 09 March 2006 (09.03.2006) entire document especiallyabstract; para [0021]-[0039]; [0074]; [0099]; [0140]; [0149]; [0224]; [0226]; [0256]-[0257]; [0274]; [0279]; [0398]	1-9, 12-14, 18, 20-28, 30-38, 40 and 42-75 ---------- 15-17, 19, 39 and 41
Y	US 6,140,343 A (DeNinno et al.) 31 October 2000 (31.10.2000) especially col 21, ln 23-33	15-16
Y	US 7,091,225 B2 (Sierra) 15 August 2006 ((15.08.2006) especially col 4, ln 06-15; col 7, ln 55-67	17, 19, 39 and 41

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2008 (24.08.2008)	30 SEP 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/68936

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-11 and 29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

APOTEX_EX1009_PGR2026-00034_Page 295